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(57) Abstract

Disclosed are novel oligosaccharide glycosides having mammalian immunosuppressive and tolerogenic properties, pharmaceutical compositions containing such oligosaccharide glycosides and methods of using such oligosaccharide glycosides to modulate cell-mediated immune responses in a mammal.

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NOVEL OLIGOSACCHARIDE GLYCOSIDES HAVING MAMMALIAN IMMUNOSUPPRESSIVE AND TOLEROGENIC PROPERTIES

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to novel oligosaccharide glycosides having mammalian immunosuppressive and/or tolerogenic properties. This invention also relates to pharmaceutical compositions comprising such oligosaccharide glycosides and to methods of using such oligosaccharide glycosides to modulate cell-mediated immune responses in a mammal.

2. References

The following references are cited in this application as superscript numbers at the relevant portion of the application:

[1] Brandley, et al., U.S. Patent No. 5,143,712, issued September 1, 1992, for "*Method of Determining a Cite of Inflammation Utilizing ELAM-1 Ligands*"

[2] Paulson, et al., International Patent Application Publication No. WO 91/19502, published 26 December 1991, for "*Intercellular Adhesion Mediators*"

[3] Lowe, et al., *Cell*, 63:475-485 (1990).

[4] Phillips, et al., *Science*, 250:1130-1132 (1990).

[5] Walz, et al., *Science*, 250:1132 et seq. (1990).

[6] Larsen, et al., *Cell*, 63:467-474 (1990).

7 Ippolito, et al., U.S. Patent Application Serial No. 07/714,161, filed June 10, 1991 for "*Immunosuppresive and Tolerogenic Oligosaccharide Glycosides*".

8 Ippolito, et al., U.S. Patent Application Serial No. 07/889,017, filed May 26, 1992 for "*Immunosuppresive and Tolerogenic Oligosaccharide Glycosides*".

9 Ippolito et al., U.S. Patent Application Serial No. 08/081,214, filed June 23, 1993 for "*Time Dependent Administration of Oligosaccharide Glycosides Related to Blood Group Determinants Having a Type I or Type II Core Structure in Reducing Inflammation in a Sensitized Mammal Arising From Exposure to an Antigen*".

10 Ekborg, et al., *Carbohydr. Res.*, 110:55-67 (1982).

11 Dahmen, et al., *Carbohydr. Res.*, 118:292-301 (1983).

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14 Paulsen, et al., *Carbohydr. Res.*, 104:195-219 (1982).

15 Chernyak, et al., *Carbohydr. Res.*, 128:269-282 (1984).

16 Fernandez-Santana, et al., *J. Carbohydr. Chem.*, 8:531-537 (1989).

17 Lee, et al., *Carbohydr. Res.*, 37:193 et seq. (1974).

18 Finan, et al., *J. Chem. Soc.*, 3089 (1962).

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21 Lemieux, et al., *Can. J. Chem.*, 57:1244 (1979).

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23 Srivastava, et al., U.S. Patent Application Serial No. 08/343,020, filed November 21, 1994 for "*Process for the Synthesis of 3'-Substituted Lewis^{x} Compounds*".

[24] Banoub, et al., *Can. J. Chem.*, 57:2091 (1979).

[25] Okamoto, et al., *Tetrahedron*, 46(17):5835-5837 (1990).

[26] Abbas, et al., *Proc. Japanese-German Symp. Berlin*, pp. 20-21 (1988).

[27] Paulsen, *Agnew. Chem. Int. Ed. Eng.*, 21:155-173 (1982).

[28] Schmidt, *Agnew. Chem. Int. Ed. Eng.*, 25:212-235 (1986).

[29] Fűgedi, et al., *Glycoconj. J.*, 4:97-108 (1987).

[30] Kameyama, et al., *Carbohydr. Res.*, 209:C_1-C_4 (1991).

[31] Ratcliffe, et al., U.S. Patent No. 5,079,353, issued January 7, 1992 for "*Sialic Acid Glycosides, Antigens, Immunoadsorbents, and Methods for Their Preparation*"

[32] Ippolito, et al., U.S. Patent Application Serial No. 08/081,212, filed June 25, 1993 for "*Immunosuppressive and Tolerogenic Modified Lewis^C and LacNAc Compounds*"

[33] Luengo, et al., *Tetrahedron Lett.*, 33:6911 (1992)

[34] Carlsen, et al., *J. Org. Chem.*, 46:3936 (1981)

[35] Unverzagt, et al., *J. prakt. Chem.*, 334:570-578 (1992)

[36] Deter-Jusynski, et al., *Carbohydr. Res.*, 28:61-74 (1973)

The disclosures of each of the above-referenced publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

3. State of the Art

A number of sialylated and sialylated/fucosylated oligosaccharide glycosides have been proposed as mediators of cell adhesion in that they

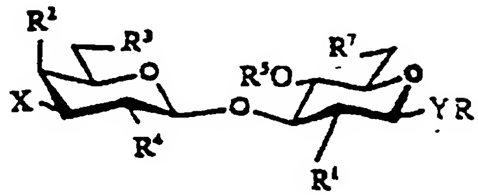
are ligands for selectins (or LEC-CAM's)[3,4,5,6]. Such selectins, including E-selectin, L-selectin and P-selectin, have been implicated as playing a seminal role in cell mediated inflammatory conditions and, heretofore, it has been postulated in the art that ligands for such selectins would possess anti-inflammatory properties[1,2]. In this regard, sialylated, fucosylated, and sialylated and fucosylated oligosaccharide structures relating to blood group determinants having a type I or a type II core structure, including Lewis^{X}, Lewis^{A}, sialyl Lewis^{X} and sialyl Lewis^{A}, have been shown by Ippolito et al.[7,8] to possess *in vivo* immunomodulating and tolerogenic properties in mammals including anti-inflammatory immunomodulating properties. Additionally, modified Lewis^{X}-OR, Lewis^{C}-OR and LacNAc-OR compounds having a sulfate group, a phosphate group or a carboxylate containing group at the 2, 3 and/or 6-positions of the galactose unit have also been disclosed to possess immunosuppressive and tolerogenic properties.[9,23] In addition, synthetic procedures for the preparation of such modified compounds have been reported.[23]

Notwithstanding these disclosures in the art, additional carbohydrate structures having immunomodulating and tolerogenic properties in mammals would provide valuable structure activity relationships and, in their own right, would be useful in providing anti-inflammatory immunomodulating properties.

SUMMARY OF THE INVENTION

This invention provides novel oligosaccharide glycosides which are useful for modulating cell mediated immune responses in a mammal, including cell mediated and immune directed inflammatory responses to an antigen in a sensitized mammal.

The oligosaccharide glycosides of this invention are represented by Formula I and II below:



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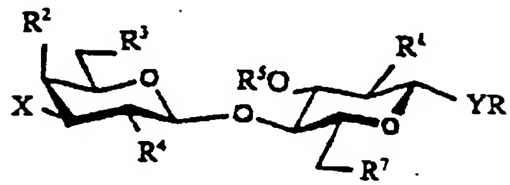

II

wherein:

Y is oxygen, sulfur or -NH-;

R is an aglycon of at least 1 carbon atom;

R^1 is selected from the group consisting of -OH, NH_2, $-N_3$, $-NHC(O)R^6$, and -fuc(C)amido, wherein R^6 is selected from the group consisting of

alkyl of from 1 to 6 carbon atoms,

cycloalkyl of from 3 to 8 carbon atoms,

aryl of from 6 to 14 carbon atoms,

alkaryl of from 7 to 20 carbon atoms,

heteroaryl of from 2 to 5 carbon atoms and from 1 to 3 hetero atoms selected from the group consisting of nitrogen, sulfur and oxygen,

substituted alkyl of from 1 to 6 carbon atoms having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkylamine of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and $-C(O)R^7$ where R^7 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms,

substituted aryl of from 6 to 14 carbon atoms having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkyl of from 1 to 6 carbon atoms, alkoxy of from

1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^6 where R^6 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms, and

substituted alkaryl having from 7 to 20 carbon atoms and having one to three substituents on the aryl moiety independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkyl of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^7 where R^7 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms,

substituted heteroaryl of from 2 to 5 carbon atoms and from 1 to 3 hetero atoms selected from the group consisting of nitrogen, sulfur and oxygen having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkyl of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^7 where R^7 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms;

R^2 is selected from the group consisting of hydrogen, hydroxyl, halo, $-OSO_3H$ and $-OP(O)(OH)_2$;

R^3 is selected from the group consisting of hydrogen, hydroxyl, halo, $-OSO_3H$ and $-OP(O)(OH)_2$;

R^4 is selected from the group consisting of hydroxyl, halo and -O-L-fucosyl;

R^5 is selected from the group consisting of hydrogen, L-fucose and L-fucose substituted at the 2, 3, and/or 4-positions with a substituent selected from the group consisting of halo, hydrogen, alkoxy, $-OSO_3H$ and $-OP(O)(OH)_2$;

R^7 is selected from the group consisting of hydrogen, hydroxy, $-OS(O)_3H$, $-OP(O)(OH)_2$, halo, azido, $-NH_2$, -NHC(O)R^6, and -fuc(C)amido, wherein R^6 is selected from the group consisting of

alkyl of from 1 to 6 carbon atoms,

cycloalkyl of from 3 to 6 carbon atoms,

aryl of from 6 to 14 carbon atoms,

alkaryl of from 7 to 20 carbon atoms,

heteroaryl of from 1 to 8 carbon atoms and from 1 to 4 hetero atoms selected from the group consisting of nitrogen, sulfur and oxygen,

heterocyclic of from 1 to 8 carbon atoms and from 1 to 4 hetero atoms selected from nitrogen, sulfur or oxygen within the ring

substituted alkyl of from 1 to 6 carbon atoms having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkylamine of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^7 where R^7 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms,

substituted aryl of from 6 to 14 carbon atoms having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkyl of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^7 where R^7 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms, and

substituted alkaryl having from 7 to 20 carbon atoms and having one to three substituents on the aryl moiety independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkyl of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^7 where R^7 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms,

substituted heteroaryl of from 2 to 5 carbon atoms and from 1 to 3 hetero atoms selected from the group consisting of nitrogen, sulfur and oxygen having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkyl of from 1 to

6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and $-C(O)R^7$ where R^7 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms;

and X is selected from the group consisting of hydroxyl, chloro, $-OSO_3H$ or $-OP(O)(OH)_2$,

and pharmaceutically acceptable salts thereof

with the proviso that when R^2 is hydroxyl, R^1 is -OH, NH_2, $-N_3$ or $-NHC(O)R^6$ where R^6 is alkyl of from 1 to 4 carbon atoms, R^5 is hydrogen, L-fucosyl, 4-sulfo-L-fucosyl or 4-phospho-L-fucosyl, R^7 is hydrogen, hydroxy, $-OS(O)_3H$, azido, $-NH_2$, $-NHC(O)R^6$ where R^6 is alkyl of from 1 to 4 carbon atoms, and X is hydroxy, $-OSO_3H$ or $-OP(O)OH_2$ and pharmaceutical salts thereof, then R^3 is not hydroxyl, $-OSO_3H$ or $-OP(O)(OH)_2$ and pharmaceutical salts thereof.

In particular, the compounds disclosed in either International Patent Application Nos. WO 92/22564 and WO 93/24506 are specifically excluded from the compounds of formula I and II of this application.

Preferably, R is an aglycon of from 1 to 20 carbon atoms, more preferably R is an aglycon of from 1 to 10 carbon atoms and still more preferably is selected from the group consisting of $-(CH_2)_8COOCH_3$, $-(CH_2)_5OCH_2CH=CH_2$ and $-(CH_2)_8CH_2OH$.

Preferably, R^1 is fuc(C)amindo or $-NHC(O)R^6$ where R^6 is alkyl containing 1 to 4 carbon atoms, cycloalkyl containing 5 or 6 carbon atoms, phenyl, substituted phenyl having one or two substituents independently selected from acetyl, nitro, or amino. More preferably, R^1 is $-NHC(O)R^6$ where R^6 is phenyl, *o*-nitrophenyl and *p*-nitrophenyl.

R^2 is preferably selected from the group consisting of hydroxyl, chloro and hydrogen. More preferably, R^2 is hydroxyl or hydrogen.

Preferably, R^3 is selected from the group consisting of hydroxy, chloro and deoxy. More preferably, R^3 is chloro or deoxy.

R^5 is preferably L-fucose.

X is preferably $-OSO_3H$, $-OP(O)OH)_2$ or a pharmaceutically acceptable salt thereof. More preferably, X is $-OSO_3H$ or a pharmaceutically acceptable salt thereof.

Preferred compounds for use in this invention include, by way of example,

8-methoxycarbonyloctyl-2-benzamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-*p*-nitrobenzamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-*o*-acetylbenzamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-cyclohexamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-fuc(C)amido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-*p*-nitrobenzamido-4-*O*-(β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-4-*O*-(4-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside

8-methoxycarbonyloctyl-4-*O*-(4-*O*-phospho-β-D-galactopyranosyl)-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3,4,6-tri-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[6-chloro-6-deoxy-3-*O*-sulfo-β-D-galactopyranosyl)]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[6-deoxy-3-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-*O*-(α-L-fucopyranosyl)-4-*O*-[4-chloro-4-deoxy-3-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-*O*-α-L-fucopyranosyl)-4-*O*-[4,6-dichloro-4,6-dideoxy-3-*O*-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl azide

2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl amine

2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl benzamide

2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl *p*-nitrobenzamide

2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl butyramide

2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl acetamide

2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl stearamide

2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl L-serine

2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside

8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-O-(4-O-sulfo-α-L-fucopyranosyl)-4-O-(3-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside

8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(3-*O*-sulfo-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside

8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(3-*O*-methyl-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside

phenylalanine amido-2-(fuc(C)-amido)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-β-D-glucopyranoside

2-benzamido-2-deoxy-3-*O*-(4-deoxy-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranosyl benzamide

cyclohexylalanine amido-2-(fuc(C)-amido)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-β-D-glucopyranoside

tryptophanamido-2-fuc(C)-amido-4-O-(3-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside

acetamido-2-(fuc(C)-amido-4-O-[3-O-sulfo-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside

benzamido 2-(fuc(C)-amido)-4-O-[3-O-sulfo-β-D-galactopyranosyl])-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-6-O-sulfo-β-D-glucopyranoside

8-methoxycarbonyloctyl 2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dichloro-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-6-benzamido-2,6-dideoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyronosyl)-4-O-[4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-6-benzamido-2,6-dideoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-6-amino-2,6-dideoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galacto-pyranosy]-6-benzamido-2,6-dideoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galacto-pyranosyl]-6-chloro-2,6-dideoxy-β-D-glucopyranoside

2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dichloro-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranosyl azide

2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranosyl azide

2-benzamido-2-deoxy-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-β-D-glucopyranoside benzamide

8-methoxycarbonyloctyl-2-amino-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dichloro-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-benzamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dichloro-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-benzamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-amino-4-O-(α-L-fucopyranosyl)-3-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-benzamido-4-O-(α-L-fucopyranosyl)-3-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-*p*-nitrobenzamido-4-O-(α-L-fucopyranosyl)-3-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

8-methoxycarbonyloctyl-2-(fuc(c)-amido)-4-O-(3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside

as well as pharmaceutically acceptable salts thereof and further including the above compounds wherein the aglycon is replaced by an aglycon of from 1 to 20 carbon atoms.

This invention further provides a pharmaceutical composition suitable for administration to a mammal which composition comprises a pharmaceutically inert carrier and an effective inflammation-reducing amount of an oligosaccharide glycoside of Formula I or II.

This invention additionally provides a method of reducing antigen-induced inflammation in a mammal which method comprises administering to said mammal an effective inflammation-reducing amount of a oligosaccharide glycoside of Formula I or II.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-D illustrates a reaction scheme for the synthesis of 2-substituted 3'-sulfo Lewis^X-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 2 illustrates a reaction scheme for the synthesis of 2-amido derivatives of lactosamine-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 3A and B illustrates a reaction scheme for the synthesis of 4'-sulfo and 4'-phospho-lactose-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 4 illustrates a reaction scheme for the synthesis of 3',6'-disulfo and 3',6'-diphospho-Lewis^C-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 5 illustrates a reaction scheme for the synthesis of 3',4',6'-trisulfo-Lewis^X-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 6 illustrates a reaction scheme for the synthesis of 3'-sulfo Y-tetrasaccharide-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 7A-C illustrates a reaction scheme for the synthesis of 6'-chloro and 6'-deoxy-3'-sulfo-Lewis^X-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 8A-C illustrates a reaction scheme for the synthesis of 4'-chloro and 4'-deoxy-3'-sulfo-Lewis^X-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 9A-C illustrates a reaction scheme for the synthesis of 4',6'-dichloro and 4',6'-dideoxy-3'-sulfo-Lewis^X-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 10 illustrates a reaction scheme for the synthesis of 4'-deoxy-3'-sulfo-Lewis^X-OR compounds [R = $-(CH_2)_8CO_2CH_3$].

Figure 11A and B illustrates a reaction scheme for the synthesis of 1-azido-3'-sulfo-Lewis^X compounds which can then be derivatized to 3'-sulfo-Lewis^X-NHR compounds.

Figure 12A and B illustrates a reaction scheme for the synthesis of 8-methoxycarbonyl 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside (Gr = -$(CH_2)_8COOCH_3$).

Figure 13A and B illustrates a reaction scheme for the synthesis of 8-methoxycarbonyl 2-acetamido-2-deoxy-3-*O*-(4-*O*-sulfo-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside.

Figure 14A and B illustrates a reaction scheme for the synthesis of 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(3-*O*-sulfo-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside.

Figure 15A and B illustrates a reaction scheme for the synthesis of 8-methoxycarbonyloctyl 2-*N*-acetamido-2-deoxy-3-*O*-(3-*O*-methyl-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside.

Figures 16A and B illustrates a reaction scheme for the synthesis of 2-benzamido-2-deoxy-3-*O*-(4-deoxy-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranosyl benzamide.

Figures 17-37 illustrate reaction schemes for the synthesis of compounds **301-423**.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

This invention is directed to novel oligosaccharide glycosides having mammalian immunosuppressive and tolerogenic properties.

However, prior to discussing this invention in further detail, the following terms will first be defined.

Definitions

As used herein the following terms have the definitions given below:

The term "cell-mediated immune response in a mammal" refers to those mammalian immune responses which are mediated by cell-cell interactions. Included within this term are cell mediated inflammatory responses to an antigen such as DTH responses as well as cell-mediated inflammatory responses arising from injury such as frost-bite injury, reperfusion injury, adult respiratory distress syndrome, and the like. Preferably, the cell-mediated immune response is a leucocyte-mediated response.

The term "antigen" refers to any protein, peptide, carbohydrate, nucleic acid or other non-endogenous substance which when exposed to a mammal induces an immune response in that mammal.

Disease conditions caused by antigen exposure include, by way of example, psoriasis, asthma, dermatitis, rheumatoid arthritis, delayed type hypersensitivity, inflammatory bowel disease, multiple scelorsis, viral pneumonia, bacterial pneumonia, and the like.

The term "sensitized mammal" or "antigen-sensitized mammal" refers to those mammals which have been previously exposed to an antigen and, accordingly, their immune systems have become educated to that antigen. Typically, initial exposure of an antigen to a mammal primes or educates the mammal's immune response to later exposure to that antigen with minimal inflammation during such initial exposure.

The term "secondary immune response" refers to the effector phase of a mammal's immune response to an antigen to which it has been

previously been sensitized. A mammal's secondary immune response is typically accompanied by inflammation at the point of antigen exposure.

The term "period for maximal inflammation" refers to the period of time typically required to achieve maximal inflammation to a specific antigen exposure. This period of time depends on several factors such as the specific antigen to which the mammal has been exposed, the particular mammalian species exposed to the antigen, etc. Accordingly, the period of time required to effect maximal antigen induced inflammation in a sensitized mammal will vary for, by way of example, asthma as opposed to rheumatoid arthritis.

The term "Lewis^X" or "Le^X" refers to the trisaccharide βGal(1→4)αFuc(1→3)βGlcNAc.

The term "Lewis^c" or "Le^c" refers to the disaccharide βGal(1→3)βGlcNAc.

The term "aglycon of at least one carbon atom" refers to non-saccharide containing residues having at least one carbon atom. Preferably, the aglycon moiety, R, has from 1 to 20 carbon atoms or is selected from the group consisting of -(A)-Z wherein A represents a covalent bond, an alkylene group of from 2 to 10 carbon atoms, a moiety of the formula $-(CH_2CR^8R^8)_n-$, and a moiety of the formula $-(CH_2CR^8R^8G)_n-$ wherein n is an integer equal to 1 to 5, each R^8 is independently selected from the group consisting of hydrogen, alkyl of from 1 to 6 carbon atoms, phenyl and phenyl substituted with 1 to 3 substituents selected from the group consisting of amine, hydroxyl, halo, alkyl of from 1 to 4 carbon atoms and alkoxy of from 1 to 4 carbon atoms and G is selected from the group consisting of -O-, -S- and -NH-; and Z is selected from the group consisting of hydrogen, methyl, phenyl, nitrophenyl, aminophenyl and,

when G is not oxygen, sulfur or nitrogen and A is not a bond, then Z is also selected from the group consisting of -OH, -SH, $-NH_2$, $-NHR^9$, $-N(R^9)_2$, -C(O)OH, $-C(O)OR^9$, $-C(O)NH-NH_2$, $-C(O)NH_2$, $-C(O)NHR^9$, $-C(O)N(R^9)_2$, and $-OR^{10}$ wherein each R^9 is independently alkyl of from 1 to 4 carbon atoms and R^{10} is an alkenyl group of from 3 to 10 carbon atoms.

Numerous aglycons are known in the art. For example, an aglycon comprising a para-nitrophenyl group (i.e., $-YR = -OC_6H_4$-p-NO_2) has been disclosed by Ekborg, et al.[10] At the appropriate time during synthesis, the nitro group is reduced to an amino group which can be protected as N-trifluoroacetamido. The trifluoroacetamido group can later be removed thereby unmasking the amino group which can then be used to further functionalize the aglycon group.

An aglycon containing sulfur is disclosed by Dahmen, et al.[11]. Specifically, this aglycon is derived from a 2-bromoethyl group which, in a substitution reaction with thionucleophiles, has been shown to lead to aglycons possessing a variety of terminal functional groups such as $-YCH_2CH_2SCH_2CO_2CH_3$ and $-YCH_2CH_2SC_6H_4$-p-NH_2.

Rana, et al.[12] discloses a 6-trifluoroacetamidohexyl aglycon ($-Y-(CH_2)_6-NHCOCF_3$) in which the trifluoroacetamido protecting group can be removed unmasking the primary amino group which can then be used to further functionalize the aglycon group.

Other exemplification of known aglycons include the 7-methoxycarbonyl-3,6-dioxaheptyl aglycon[13] ($-YCH_2-CH_2)_2OCH_2CO_2CH_3$; the 2-(4-methoxycarbonylbutanecarboxamido)ethyl[14] ($-YCH_2CH_2NHC(O)(CH_2)_4CO_2CH_3$); and the allyl aglycon[15]

($-YCH_2CH=CH_2$) which, by radical co-polymerization with an appropriate monomer, leads to co-polymers; other allyl aglycons[16] are known [e.g., $-Y(CH_2CH_2O)_2CH_2CH=CH_2$]. Additionally, allyl aglycons can be derivatized in the presence of 2-aminoethane-thiol[17] to provide for aglycons $-YCH_2CH_2CH_2SCH_2CH_2NH_2$. Still other aglycons are illustrated hereinbelow.

Additionally, as shown by Ratcliffe et al.[31], the R group can be an additional saccharide-OR^{11} or an oligosaccharide-OR^{11} containing an aglycon at the reducing sugar terminus.

Still further, when Y is -NH-, the aglycon can be the residue of an amino acid or a peptide of from 2 to 10 amino acids in length. Amino acids include any of the naturally occurring amino acids, as well as synthetic analogs and derivatives thereof. α-Amino acids comprise a carbon atom to which is bonded an amino group, a carboxyl group, a hydrogen atom, and a distinctive group referred to as a "side chain". The side chains of naturally occurring amino acids are well known in the art and include, for example, hydrogen (e.g., as in glycine), alkyl (e.g., as in alanine, valine, leucine, isoleucine, proline), substituted alkyl (e.g., as in threonine, serine, methionine, cysteine, aspartic acid, asparagine, glutamic acid, glutamine, arginine, and lysine), arylalkyl (e.g., as in phenylalanine and tryptophan), substituted arylalkyl (e.g., as in tyrosine), and heteroarylalkyl (e.g., as in histidine).

One of skill in the art will appreciate that the term "amino acid" can also include β-, γ-, δ-, and ω-amino acids, and the like. Unnatural amino acids are also known in the art, as set forth in, for example, Williams (ed.), *Synthesis of Optically Active α-Amino Acids*, Pergamon Press (1989); Evans et al., *J. Amer. Chem. Soc.*, 112:4011-4030 (1990); Pu et al., *J. Amer. Chem. Soc.*, 56:1280-1283 (1991); and Williams et al., *J. Amer.*

Chem. Soc., 113:9276-9286 (1991) which are incorporated herein by reference in their entirety.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. Amino acid residues are abbreviated as follows: Phenylalanine is Phe or F; Leucine is Leu or L; Isoleucine is Ile or I; Methionine is Met or M; Norleucine is Nle; Valine is Val or V; Serine is Ser or S; Proline is Pro or P; Threonine is Thr or T; Alanine is Ala or A; Tyrosine is Tyr or Y; Histidine is His or H; Glutamine is Gln or Q; Asparagine is Asn or N; Lysine is Lys or K; Aspartic Acid is Asp or D; Glutamic Acid is Glu or E; Cysteine is Cys or C; Tryptophan is Trp or W; Arginine is Arg or R; Glycine is Gly or G, and X is any amino acid. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α,α-disubstituted amino acids, N-alkyl amino acids, and other unconventional amino acids are also suitable components for compounds described herein. Examples of unconventional amino acids include: 4-hydroxyproline, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

Preferably, the aglycon moiety is a hydrophobic group and most preferably, the aglycon moiety is a hydrophobic group selected from the group consisting of $-(CH_2)_8COOCH_3$, $-(CH_2)_5OCH_2CH=CH_2$ and $-(CH_2)_8CH_2OH$.

The term "oligosaccharide" refers to a carbohydrate structure having from 2 to about 7 saccharide units. The particular saccharide units

employed are not critical and include, by way of example, all natural and synthetic derivatives of glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, fucose, sialic acid, 3-deoxy-D,L-octulosonic acid, and the like. In addition to being in their pyranose form, all saccharide units described herein are in their D form except for fucose which is in its L form.

The term "pharmaceutically acceptable salts" includes the pharmaceutically acceptable addition salts of the compounds of Formula I or Formula II derived from a variety of organic and inorganic counter salts well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetralkylammonium, chloride, fluoride, bromide, hydroxide and the like.

The term "sulfate" refers to the $-O-S(O_2)-OH$ group, which readily forms pharmaceutically acceptable salts thereof.

The term "phosphate" refers to the group $-O-P(O)-(OH)_2$, which readily forms pharmaceutically acceptable salts thereof (e.g., $-O-P(O)-(O^-Na^+)_2$).

The term "removable blocking group" or "blocking group" or "protecting group" refers to any group which when bound to one or more hydroxyl and/or amine groups of oligosaccharide glycoside compounds and oligosaccharide glycoside related compounds prevents reactions from occurring at these hydroxyl and/or amine groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl or amine group. Typically, the particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as benzyl, acetyl, benzoyl, chloroacetyl, benzylidene, *t*-butyldiphenylsilyl,

t-butyldimethylsilyl and any other group that can be introduced either enzymatically or chemically onto a hydroxyl functionality and later selectively removed either by enzymatic or chemical methods in mild conditions compatible with the nature of the product. Preferred amine blocking groups include these well known in the art such as carboxybenzyloxy (CBZ), *t*-butyloxy carbonyl (*t*-Boc), and any other group that can be introduced either enzymatically or chemically onto an amine functionality and later selectively removed either by enzymatic or chemical methods in mild conditions compatible with the nature of the product.

"Alkyl" refers to monovalent alkyl groups having from 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, and the like.

"Alkylene" refers to divalent alkylene groups preferably having from 1 to 10 carbon atoms and more preferably 2 to 10 carbon atoms. This term is exemplified by groups such as methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), the propylene isomers (e.g., $-CH_2CH_2CH_2-$ and $-CH(CH_3)CH_2-$) and the like.

"Alkaryl" refers to -alkylene-aryl groups of from 7 to 20 carbon atoms preferably having from 1 to 10 carbon atoms in the alkylene moiety and from 6 to 10 carbon atoms in the aryl moiety. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

"Alkoxy" refers to the group "alkyl-O-". Preferred alkoxy groups include, by way of example, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

"Aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

"Cycloalkyl" refers to cyclic alkyl groups of from 3 to 8 carbon atoms having a single cyclic ring or multiple condensed rings which can be optionally substituted with from 1 to 3 alkyl groups. Preferred cycloalkyl groups include those having from 3 to 6 carbon atoms. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, 1-methylcyclopropyl, 2-methylcyclopentyl, 2-methylcyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

"Halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is either chloro or bromo.

"Heteroaryl" refers to a monovalent aromatic carbocyclic group of from 1 to 8 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within the ring. Preferred heteroaryl groups have from 2 to 5 carbon atoms and 1 to 3 heteroatoms selected from oxygen, nitrogen and sulfur.

"Heterocycle" or "heterocyclic" refers to a monovalent saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 8 carbon atoms and from 1 to 4 hetero atoms selected from nitrogen, sulfur or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 3 substituents selected from the group consisting of alkyl, alkoxy, aryl,

aryloxy, halo, nitro, heteroaryl, thioalkoxy, thioaryloxy and the like. Such heterocyclic groups can have a single ring or multiple condensed rings.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline and the like.

"Thiol" refers to the group -SH.

"Thioalkoxy" refers to the groups -S-alkyl.

General Synthetic Methodology

Chemical methods for the synthesis of the oligosaccharides are known in the art. These oligosaccharides are generally assembled using suitably protected individual monosaccharides and/or suitably protected individual disaccharides intermediates.

The specific methods employed are generally adapted and optimized for each individual structure to be synthesized. In general, the chemical synthesis of all or part of these oligosaccharides first involves formation of a glycosidic linkage on the anomeric carbon atom of the reducing sugar. Specifically, an appropriately protected form of a naturally occurring or of a chemically modified glucose structure (the glycosyl donor) is selectively modified at the anomeric center of the reducing unit so as to introduce a

leaving group comprising halides, trichloroacetimidate, acetyl, thioglycoside, etc. The donor is then reacted under catalytic conditions well known in the art with an aglycon or an appropriate form of a carbohydrate acceptor which possess one free hydroxyl, free thiol or primary/secondary amino group at the position where the glycosidic linkage is to be established. A large variety of aglycon moieties are known in the art and can be attached with the proper configuration to the anomeric center of the reducing unit. Appropriate use of compatible blocking groups, well known in the art of carbohydrate synthesis, will allow selective modification of the synthesized structures or the further attachment of additional sugar units or sugar blocks to the acceptor structures.

After formation of the glycosidic linkage, the saccharide glycoside can be used to effect coupling of additional saccharide unit(s) or chemically modified at selected positions or, after conventional deprotection, used in an enzymatic synthesis. In general, chemical coupling of a naturally occurring or chemically modified saccharide unit to the saccharide glycoside is accomplished by employing established chemistry well documented in the literature. See, for example, Okamoto et al.[25], Abbas et al.[26], Paulsen[27], Schmidt[28], Fugedi et al.[29], Kameyama et al.[30] and Ratcliffe, et al.[31]

The figures attached hereto illustrate a variety of complete chemical synthetic schemes used for preparing representative oligosaccharide glycosides of this invention. By necessity, these schemes illustrate the synthesis of specific compounds of this invention. However, it is well within the skill of those in the art to adapt these synthetic schemes to prepare any of the compounds of this invention. Moreover, it will be appreciated by those skilled in the art that where typical or preferred process conditions (e.g., reaction temperatures, times, mole ratios of

reactants, solvents, pressures, etc.) are given, other process conditions may also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvents used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

A. 2-Amino and 2-Amido 3'-Sulfo Lewis^x-OR Derivatives

Figure 1 illustrates the synthesis of 2-substituted 3'-sulfo Lewis^X derivatives. As shown in this figure, 3'-sulfo-Lewis^X derivatives having an amino or an amido substituent at the 2-position are prepared beginning with 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide (1'), the synthesis of which is known in the art[19-20].

Compound 1' is first converted to the aglycon by reaction with from about 1.1 to about 5 equivalents of HYR, e.g., $HO(CH_2)_8COOCH_3$, by well known chemistry. In a preferred embodiment, compound 1' is reacted with 8-methoxycarbonyloctanol in anhydrous dichloromethane containing molecular sieves and a catalyst, such as silver carbonate, to provide intermediate 1" in 90% yield as a crystalline solid. This reaction is generally conducted at a temperature ranging from -20°C to -10°C for a period of 3 to 4 hours.

The 3, 4 and 6 hydroxyl groups of compound 1" are then deprotected by reaction of compound 1" with sodium methoxide in methanol to give 8-methoxycarbonyloctyl-2-azido-2-deoxy-β-D-glucopyranoside (1a).

The 4 and 6 hydroxy groups of compound 1a are then protected as the 4,6-O-benzylidene derivative by reaction of compound 1a with benzaldehyde dimethylacetal in the presence of an acid catalyst, such as *p*-toluenesulfonic acid, to give compound 1. This reaction is typically

conducted at room temperature in an anhydrous inert solvent, such as acetonitrile, and is generally complete in about 0.5 to 5 hours.

The unprotected hydroxyl group of compound **1** is then glycosylated with *p*-chlorophenyl tribenzyl thiofucose (**2**). Compound **2** is well known in the art and may be prepared by the procedures described by Srivastava et al.[23] The glycosylation reaction is typically conducted by contacting compound **1** with about 1 to 2 equivalents, preferably 1.2 equivalents, of compound **2** in the presence of a cupric bromide/dimethylformamide catalyst and tetraethylammonium bromide. This reaction is generally conducted at room temperature in an anhydrous inert solvent, such dichloromethane, containing molecular sieves. The reaction is typically complete after about 7 to 15 hours and provides compound **3**.

The 4,6-O-benzylidene group of compound **3** is then opened regioselectively to provide compound **4** having an unblocked hydroxyl group at the 4 position. Regioselective opening of the benzylidene group is affected by treatment of compound **3** with at least a molar equivalent, preferably an excess, of sodium cyanoborohydride or a similar hydride reducing agent, in the presence of an ethereal solution of hydrochloric acid. The reaction is typically conducted in a suitable inert solvent, such as tetrahydrofuran, and is preferably maintained under anhydrous conditions by, for example, the inclusion of molecular sieves. A pH indicator, such a methyl orange, is generally added to the reaction system and the reaction is generally conducted at a pH of about 3 or less. The reaction conditions are not critical and the conditions are selected so as to produce compound **4**. In a preferred embodiment, about 2 to about 20, preferably 5 to 10 equivalents of sodium cyanoborohydride is employed at a reaction temperature of from about -15°C to about 20°C (preferably 0°C) for a period of from about 1 to about 7 hours. The reaction generally provides compound **4** in about 60% yield.

Compound 4 is next converted to 8-methoxycarbonyloctyl-2-azido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyransoyl)-4-O-(β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (7) by reaction with from about 1.1 to about 2 equivalents of O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-trichloroacetimidate (5) using conventional coupling conditions, followed by deacetylation of the resulting trisaccharide.

The coupling reaction is preferably conducted using an excess of boron trifluoride etherate relative to the galactose imidate and preferably using from about 1.1 to about 2 equivalents. The reaction is typically conducted at from about -30°C to about 10°C (preferably -10°C to 0°C) in a suitable anhydrous organic solvent such as dichloromethane or a 1:2 mixture of dichloromethane:ether.

Deacetylation is conducted using conventional reaction conditions, preferably using sodium methoxide in methanol, to provide compound 7.

The galactose unit of compound 7 is next converted to the 4,6-O-benzylidene derivative compound 8 by reaction of compound 7 with from about 1 to about 2 equivalents of benzaldehyde dimethylacetal. This reaction is preferably conducted in an inert organic solvent, such as acetonitrile, in the presence of an acidic catalyst, such as *p*-toluenesulfonic acid (pTSA). In a preferred embodiment, the reaction is conducted at a temperature of from about 0°C to about 35°C (preferably 15°C to 25°) for from about 1 to about 5 hours. Standard work-up procedures provide compound 8 in about 76% yield.

The 2-azido group of compound 8 is then reduced to an amino group by contacting compound 8 with a saturated solution of hydrogen sulfide in a suitable solvent. In a preferred embodiment, the solvent comprises pyridine (2 parts), triethylamine (0.05 parts) and water (0.05

parts). This reaction is preferably conducted initially at 0°C for about 2 hours and then at room temperature for about 5 to 15 hours, preferably for about 15 hours, to provide the amino derivative **9**.

The amino group of 8-methoxycarbonyloctyl-2-amino-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**9**) can then be acylated to form various 2-amido derivatives. Suitable acylating agents for use in this reaction include acyl halides, such as carboxylic acid chlorides and bromides; carboxylic acid anhydrides and carboxylic acids.

Preferred acylating agents are those having the formula W-C(O)R^6 where W is chloro or hydroxyl and R^6 is selected from the group consisting of alkyl of from 1 to 6 carbon atoms, cycloalkyl of from 3 to 8 carbon atoms, heterocyclic, aryl of from 6 to 14 carbon atoms, alkaryl of from 7 to 20 carbon atoms, heteroaryl of from 1 to 8 carbon atoms and from 1 to 4 hetero atoms selected from the group consisting of nitrogen, sulfur and oxygen, substituted alkyl of from 1 to 6 carbon atoms having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkylamino of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^6 where R^6 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms, substituted aryl of from 6 to 14 carbon atoms having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkyl of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^6 where R^6 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms, and substituted alkaryl having from 7 to 20 carbon atoms and having one to three substituents on the aryl moiety independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino,

alkyl of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^6 where R^6 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms, substituted heteroaryl of from 2 to 5 carbon atoms and from 1 to 3 hetero atoms selected from the group consisting of nitrogen, sulfur and oxygen having one to three substituents independently selected from the group consisting of halo, nitro, cyano, carboxyl, amino, alkyl of from 1 to 6 carbon atoms, alkoxy of from 1 to 6 carbon atoms, thiol, hydroxyl, thioalkoxy of from 1 to 6 carbon atoms and -C(O)R^6 where R^6 is selected from the group consisting of hydrogen and alkyl of from 1 to 6 carbon atoms; is an alkyl group of from 1 to 6 carbon atoms.

Suitable acylating agents include, by way of example only, acetyl chloride, acetic anhydride, benzoyl chloride, *p*-nitrobenzoyl chloride, *o*-acetylbenzoic acid, cyclohexanecarboxylic acid, tri-O-acetyl-fucose(C)-carboxylic acid, azealic acid, and stearic acid. Activated esters of such acids may also be employed.

When the acylating agent employed is an acyl halide, the acylation reaction is typically conducted by contacting compound **9** with about 1.5 to 5 equivalents of the acyl halide, such as benzoyl chloride, in a suitable solvent. A preferred solvent for this reaction is a mixture containing about 4 parts of methanol and 1 part of a saturated aqueous solution of sodium bicarbonate. The reaction is typically monitored by tlc and additional acyl halide may be added periodically until the reaction is complete. Generally, the reaction is conducted at a temperature of from about 0° to 22°C for a period of about 1 to about 15 hours. When benzoyl chloride is employed as the acylating agent, the product of the reaction is compound **11**; and when *p*-nitrobenzoyl chloride is employed, the product is compound **12**.

Alternatively, the 2-amino group of compound **9** can be acylated using a carboxylic acid. This reaction is typically conducted by contacting compound **9** with about 1.5 to about 5 equivalents of the carboxylic acid in the presence of about 1.5 to about 5 equivalents of a coupling agent. Suitable coupling agents include, by way of example, 1-(3-dimethylaminopropyl)-3-ethylcarbodimidate hydrochloride and dicyclohexyl carbodiimide which provide for the activated ester. The reaction is generally conducted in an anhydrous inert solvent, such as dichloromethane, at room temperature for about 0.5 to about 24 hours. During this period, the reaction is typically monitored by tlc and additional amounts of the carboxylic acid and the coupling agent may be added periodically until the reaction is complete. When the carboxylic acid employed in this reaction is ortho-acetylbenzoic acid, the product is compound **13**. When the carboxylic acid is cyclohexanecarboxylic acid or tri-O-acetyl-fucose(C)-carboxylic acid, the products are compounds **14** and **19**, respectively.

The 2-amido derivatives of 8-methoxycarbonyloctyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside, e.g. compounds **11**, **12**, **13**, **14**, and **19**, are then selectively sulfonated to provide the corresponding 3′-O-sulfo derivatives. Selective sulfonation can be achieved by contacting the trisaccharide with about 1.0 to about 3 equivalents of a sulfur trioxide/pyridine complex in pyridine. Alternatively, sulfur trioxide complexes with dimethylformamide, triethylamine, dioxane or mixtures thereof may be employed. The conditions for the sulfonation reaction are selected to favor substitution only at the 3′ position of the galactose unit which, in the case of sulfur trioxide in DMF includes reaction at from about -30°C to about -50°C; whereas for sulfur trioxide complexes with pyridine or triethylamine includes reaction temperatures of 0°C to 30°C. In any event, the reaction is maintained at this temperature

for a period of from about 1 to about 20 hours to provide for 3'-substitution of the trisaccharide derivative. The resulting 3'-O-sulfo derivatives are optionally converted to a salt thereof, e.g., compounds **15**, **16**, **17**, **18**, and **20**, by contact with a suitable cation exchange resin.

Compounds **15**, **16**, **17**, **18** and **20** or similar 2-amido 3'-O-sulfo derivatives are then deblocked using conventional deblocking methodology. The particular methodology employed is selected based on the blocking groups present in the molecule and it is well within the skill of the art to select a suitable deblocking methodology. For example, in the case of compound **15**, deblocking is achieved by hydrogenolysis of the benzyl and benzylidene protecting groups which provides for 8-methoxycarbonyloctyl-2-benzamido-3-O-(α-L-fucopyranosyl)-4-O-(3-O-sulfo-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside, which can be converted to a salt by contact with a cation exchange resin. Formation of the sodium salt, for example, provides for compound **22**. Compounds **16**, **17** and **18** are similarly deblocked. Compound **20**, which contains acetyl (Ac) protecting groups on the fuc(C)amido moiety, is first treated with sodium methoxide in methanol to remove the acetyl groups to provide for compound **21** and then hydrogenolyzed to provide 8-methoxycarbonyloctyl-2-fuc(C)amido-3-O-(α-L-fucopyranosyl)-4-O-(3-O-sulfo-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside **26**, which can be converted to a salt by contact with a cation exchange resin.

Using methodology similar to that described above for the 2-amido derivatives of 8-methoxycarbonyloctyl-3-O-(α-L-fucopyranosyl)-4-O-(3-O-sulfo-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside, the 2-amino derivative can be prepared by selectively sulfonating compound **8** using a sulfur trioxide-pyridine complex to provide compound **10** and then reducing the 2-azido group of compound **10** (while simultaneously removing the benzyl blocking groups) by hydrogenating compound **10** in the presence of

a suitable catalyst, such as 5% palladium on carbon, to provide 8-methoxycarbonyloctyl-2-amino-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside, which can be converted to a salt by contact with a cation exchange resin. Formation of the sodium salt, for example, provides for compound **27**.

B. 2-Amido Lactosamine-OR Derivatives

Figure 2 illustrates the synthesis of the 2-*p*-nitrobenzamido derivative of 8-methoxycarbonyloctyl-4-O-(β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranose (e.g., compound **30**). Specifically, in Figure 2, 8-methoxycarbonyloctyl-2-amino-4-O-(β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranose (**28**) was prepared by glycosylation of 2-azido-2-deoxy-3,6,2′,3′,4′,6′-hexa-O-acetyl-α-D-lactopyranosyl bromide (described by Lemieux, et al.[22]) with 8-methoxycarbonyloctanol followed by reduction of the azido group with H_2S in pyridine:triethylamine:water (4:1:0.1) and deacetylation. This compound is perbenzoylated with *p*-nitrobenzoyl chloride in pyridine to form intermediate **29**. The conditions for this reaction are not critical and typically the reaction is conducted at a temperature of from about -10°C to about 22°C for about 5 to about 15 hours or until all of the hydroxyl and amino groups present in the oligosaccharide glycoside reactant have been acylated.

The hydroxyl groups of intermediate **29** are then selectively deblocked using conventional procedures, preferably using sodium methoxide in methanol, to provide 8-methoxycarbonyloctyl-2-*p*-nitrobenzamido-4-O-(β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranose (**30**). By employing acyl chlorides of the general formula Cl-C(O)R^6 (R^6 is defined as above) in place of *p*-nitrobenzoyl chloride, various 2-amido lactosamine-OR derivatives can be prepared.

C. 4'-Sulfo and 4'-Phospho Lactose-OR Derivatives

Figure 3 illustrates the synthesis of the 4-O-sulfo- and 4-O-phospho-derivatives of 8-methoxycarbonyloctyl-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside. Specifically, in Figure 3, 8-methoxycarbonyloctyl lactose (**31**), prepared by the procedures described by Bundle and Banoub[24], is converted into a mixture of the 3,4-O- and 4,6-O-isopropylidene derivatives, compounds **32** and **33**, respectively, by reaction with 2,2-dimethoxypropane in the presence of an acidic catalyst, such as *p*-toluenesulfonic acid. The mixture of compounds **32** and **33** is next benzoylated with an excess of benzoyl chloride to provide compounds **34** and **35**. The isopropylidine blocking group of compounds **34** and **35** is then removed by contacting the mixture with aqueous acetic acid, preferably 80% aqueous acetic acid. The resulting diol is generally not isolated but is selectively benzoylated with benzoyl chloride at -50°C to provide for compound **36**. Compound **36** is then sulfonated using a sulfur trioxide/pyridine complex to provide the 4-O-sulfo derivative **37**, after conversion to its sodium salt. Deblocking of compound **37** using conventional procedures, e.g. treatment with sodium methoxide in methanol, provides 8-methoxycarbonyloctyl-4-O-(4-O-sulfo-β-D-galacto-pyranosyl)-β-D-glucopyranoside (**38**), after conversion to its sodium salt.

Alternatively, intermediate **36** can be phosphorylated with diphenyl chlorophosphate and dimethylaminopyridine to give compound **39**. Deblocking of the phosphate group of compound **39** by hydrogenolysis followed by deblocking of the hydroxyl groups using sodium methoxide in methanol affords 8-methoxycarbonyloctyl-4-O-(4-O-phospho-β-D-galacto-pyranosyl)-β-D-glucopyranoside (**40**), after conversion to its sodium salt.

D. 3',6'-Disulfo and 3',6'-Diphospho Lewis[c]-OR Derivatives

Figure 4 illustrates the synthesis of the 3',6'-disulfo- and 3',6'-diphospho- derivatives of 8-methoxycarbonyloctyl-2-acetamido-3-O-(β-D-

galactopyranosyl)-2-deoxy-β-D-glucopyranoside, e.g., compounds **43** and **45**. As shown in Figure 4, 8-methoxycarbonyloctyl-2-acetamido-3-O-(β-D-galactopyranosyl)-2-deoxy-4,6-O-benzylidene-β-D-glucopyranoside (**41**), prepared as described in the Examples set forth below, can be selectively disulfonated by treatment with about 2 to about 4 equivalents of a sulfur trioxide/pyridine complex in pyridine to afford the 3',6'-di-O-sulfo blocked intermediate **42**. Preferably, this reaction is conducted at a temperature of from about 0°C to about 20°C for a period of about 3 to about 15 hours. Deblocking of compound **42** using conventional hydrogenolysis conditions provides 8-methoxycarbonyloctyl-2-acetamido-3-O-(3,6-di-O-sulfo-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (**43**), after conversion to its sodium salt.

Additionally, the 3' and 6' hydroxyl groups of compound **41** can be selectively phosphorylated by treatment with about 2.0 to about 4.0 equivalents of diphenyl chlorophosphate and about 2.0 to about 4.0 equivalents of dimethylaminopyridine to provide intermediate **44**. This reaction is preferably conducted in pyridine at a temperature of from about 0°C to about 20°C for about 1 to about 5 hours. Conventional deblocking of compound **44**, e.g. hydrogenolysis, affords 8-methoxycarbonyloctyl-2-acetamido-3-O-(3,6-di-O-phospho-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (**45**), after conversion to its sodium salt.

E. 3',4',6'-Trisulfo Lewis^x-OR Derivatives

Figure 5 illustrates the synthesis of a 3',4',6'-trisulfo derivative of 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside. Specifically, in Figure 5, compound **46**, prepared by the procedure described by Srivastava et al.[23], is selectively tri-O-sulfonated by treatment with about 3.0 to about 6.0 equivalents of a sulfur trioxide/pyridine complex in pyridine to afford the 3',4',6'-tri-O-sulfo blocked intermediate **47**. This reaction is preferably

conducted at a temperature of from about 0°C to about 20° for a period of about 5 to about 15 hours. Deblocking of compound **47** using conventional hydrogenolysis conditions provides 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3,4,6-tri-O-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (**48**), after conversion to its sodium salt.

F. 3'-Sulfo Y-Tetrasaccharide-OR Derivatives

Figure 6 illustrates the synthesis of 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (**52**) beginning with blocked tetrasaccharide **49**. Compound **49** can be prepared as set forth in the Examples below. Removal of the benzoyl blocking group from compound **49** by treatment with sodium methoxide in methanol provides compound **50** having an unblocked hydroxyl group in the 3 position of the galactose unit. Sulfonation of this hydroxyl group with a sulfur trioxide/pyridine complex provides intermediate **51** which is then deblocked under conventional hydrogenolysis conditions to afford compound **52**, after conversion to its sodium salt.

G. Chloro, Dichloro, Deoxy and Dideoxy 3'-Sulfo Lewis^x-OR Derivatives

Figure 7 illustrates the synthesis of 6'-chloro and 6'-deoxy 3'-sulfo-Le^x-OR derivatives. As shown in Figure 7, the unblocked hydroxyl group of 1,2,3,4-di-O-isopropylidene-D-galactopyranose (**53**), obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, USA, is first converted into a chloro substituent by treatment of compound **53** with an inorganic acid halide, such as sulfuryl chloride, in a suitable solvent. In a preferred embodiment, compound **53** is contacted with an excess of sulfuryl chloride in pyridine. This reaction is typically conducted at a temperature in the range from about -40°C to about 20°C, preferably at -40°C, for about 1 to about 15 hours. Subsequent hydrolysis of the isopropylidene blocking groups using 90% aqueous trifluoroacetic acid followed by

acetylation of the resulting unblocked hydroxyl groups using conventional procedures, e.g., excess acetic anhydride in pyridine, affords intermediate **54**.

Compound **54** is then converted in two steps into O-(2,3,4-tri-O-acetyl-6-chloro-6-deoxy-α-D-glucopyranosyl) trichloroacetimidate (**55**). First, the anomeric acetate group of compound **54** is selectively removed by reaction with hydrazine acetate. Preferably, this reaction is conducted by contacting compound **54** with from about 1.1 to about 1.5 equivalents of hydrazine acetate (prepared by known procedures from hydrazine and acetic anhydride) at a temperature of from about 0°C to about 20°C for about 5 to about 10 hours. Typically, the reaction is conducted in an anhydrous solvent, such as dimethylformamide. Treatment of the resulting product with trichloroacetonitrile in the presence of an amine, such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), provides compound **55**. This reaction is typically conducted in an anhydrous inert organic solvent, such as dichloromethane, using an excess of trichloroacetonitrile.

Compound **55** is then coupled with 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**56**), prepared according to the procedures described by Srivastava et al.[23], using conventional coupling conditions to provide 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranoysl)-4-O-[6-chloro-6-deoxy-2,3,4-tri-O-acetyl-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**57**). The coupling reaction is generally conducted by contacting compound **56** with from about 1.1 to about 2 equivalents of O-(6-chloro-6-deoxy-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-trichloroacetimidate (**55**) and an excess of boron trifluoride etherate relative to the galactose imidate. Preferably from about 1.1 to about 2 equivalents of boron trifluoride etherate are employed. The reaction is typically conducted at from about -30°C to about 10°C

(preferably -10°C) in a suitable anhydrous organic solvent such as dichloromethane or a 1:2 mixture of dichloromethane:ether.

Compound **57** is then deacetylated using conventional reaction conditions, preferably using sodium methoxide in methanol, to provide intermediate **58**. Sulfonation of compound **58** using conventional procedures, e.g., treatment with a sulfur trioxide/pyridine complex, affords the 6′-chloro-3′-O-sulfo intermediate **59** which is deblocked using conventional hydrogenolysis conditions to provide 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[6-chloro-6-deoxy-3-O-sulfo-β-D-galactopyranosyl)]-β-D-glucopyranoside (**60**), after conversion to its sodium salt.

The 6′-deoxy-3′-sulfo-Lewis^x-OR derivative, compound **62**, is readily prepared from the corresponding blocked 6′-chloro derivative, compound **57**, by reduction (e.g. de-chlorination) with tributyltin hydride. This reaction is typically conducted by contacting compound **57** with about 1.0 to about 10.0 equivalents of tributyltin hydride, preferably about 10 equivalents, in the presence of a free radical initiator, such as azobisisobutyronitrile (AIBN). The reaction is generally conducted in an inert solvent, such as toluene, at a temperature of from about 20°C to about 80°C for a period of about 1.0 to about 10 hours. Reduction of compound **57** under these conditions affords compound **60a**.

Compound **60a** is then deacetylated, sulfonated and deblocked using conventional techniques to provide 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[6-deoxy-3-O-sulfo-β-D-galactopyranosyl)]-β-D-glucopyranoside (**63**), after conversion to its sodium salt.

Figure 8 illustrates the synthesis of 4′-chloro and 4′-deoxy 3′-sulfo-Lewis^x-OR derivatives. Specifically, in Figure 8, methyl α-D-

glucopyranoside (**64**) is selectively protected by first forming the 4,6-O-benzylidene derivative by reacting compound **64** with benzaldehyde dimethylacetal in the presence of an acidic catalyst, such as *p*-toluenesulfonic acid, and then benzoylating the benzylidene intermediate using benzoyl bromide in pyridine to provide methyl 4-6-O-benzylidene-2,3-di-O-benzyl-α-D-glucopyranoside (**65**).

The 4,6-O-benzylidene group of compound **65** is then opened regioselectively to provide methyl 6-O-benzyl-2,3-di-O-benzoyl-α-D-glucopyranoside (**66**), which has an unblocked hydroxyl group at the 4 position of the glucose unit. Regioselective opening of the benzylidene group is affected by treating compound **65** with at least a molar equivalent, preferably an excess, of sodium cyanoborohydride or a similar hydride reducing agent, in the presence of an ethereal solution of hydrochloric acid. The reaction is typically conducted in a suitable inert solvent, such as tetrahydrofuran and is preferably maintained under anhydrous conditions by, for example, the inclusion of molecular sieves. A pH indicator, such a methyl orange, is generally added to the reaction system and the reaction is generally conducted at a pH of about 3 or less. The reaction conditions are not critical and the conditions are selected so as to produce compound **66**. In a preferred embodiment, about 2 to about 20, preferably 5 to 10 equivalents of sodium cyanoborohydride are employed at a reaction temperature of from about -15°C to about 20°C (preferably 0°C) for a period of from about 1 to about 7 hours. The reaction provides compound **66** as a white solid.

Reaction of compound **66** with sulfuryl chloride in a suitable solvent, such as pyridine, then affords methyl 6-O-benzyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-α-D-glucopyranoside (**67**). This reaction is typically conducted at a temperature in the range from about -40° to about 20°C, preferably at -40°C, using an excess of sulfuryl chloride. Subsequent

acetolysis of compound **67** using acetic anhydride containing sulfuric acid provides acetyl 6-O-benzyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-α-D-glucopyranoside (**68**).

Compound **68** is next converted in two steps into α-(6-O-acetyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-α-D-galactopyranosyl)-trichloroacetimidate (**69**). First, the anomeric acetate group of compound **69** is selectively removed by reaction with hydrazine acetate. This reaction is preferably conducted by contacting compound **69** with from about 1.1 to about 1.5 equivalents of hydrazine acetate (prepared by known procedures from hydrazine and acetic anhydride) at a temperature of from about 0°C to about 20°C for about 5 to about 10 hours. Typically, the reaction is conducted in an anhydrous solvent, such as dimethylformamide. Treatment of the resulting product with trichloroacetonitrile in the presence of an amine, such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), provides compound **69**. This reaction is typically conducted in an anhydrous inert organic solvent, such as dichloromethane, using an excess of trichloroacetonitrile.

Compound **69** is then coupled with 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**56**), prepared according to the procedures described by Srivastava et al.[23], using conventional coupling conditions to provide 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-O-acetyl-2,3-di-O-benzyl-4-chloro-4-deoxy-β-D-galactopyranosyl]-2-deoxy-6-O-benzyl-β-D-glucopyranoside (**70**). The coupling reaction is generally conducted by contacting compound **56** with from about 1.1 to about 2 equivalents of trichloroacetimidate **69** and an excess of boron trifluoride etherate relative to the imidate. Preferably from about 1.1 to about 2 equivalents of boron trifluoride etherate are employed in this reaction. The reaction is typically conducted at from about -30°C to

about 10°C (preferably -10°C) in a suitable anhydrous organic solvent such as dichloromethane or a 1:2 mixture of dichloromethane:ether.

The blocking groups of compound **70** are then manipulated to allow the 3-hydroxyl group of the galactose unit to be selectively sulfonated. Specifically, the acetyl and benzoyl blocking groups of compound **70** are first removed using conventional procedures, preferably using sodium methoxide in methanol, to provide intermediate **71**. The unblocked 6-hydroxyl group of the galactose unit of compound **71** is then re-blocked using a suitable protecting group. Preferably, this hydroxyl group is blocked as the *tert*-butyldimethylsilyl (TBDMS) derivative by contacting compound **71** with excess *tert*-butyldimethylsilyl chloride in pyridine. Acylation of the remaining hydroxyl groups using conventional procedures, e.g., acetic anhydride, provides intermediate **72**. The *tert*-butyldimethylsilyl blocking group is then removed by contacting compound **72** with tetrabutylammonium fluoride hydrate under conventional deblocking conditions to afford intermediate **73**. The unblocked hydroxyl group of compound **73** is then re-blocked as the benzyl ether by reaction of compound **73** with excess benzyl bromide in the presence of silver oxide to provide compound **74**. Compound **74** is then deacetylated using convention conditions, e.g. sodium methoxide in methanol, to afford 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-O-benzyl-4-chloro-4-deoxy-β-D-galactopyranosyl]-2-deoxy-6-O-benzyl-β-D-glucopyranoside (**75**).

Compound **75** is then sulfonated selectively on the 3-hydroxyl group of the galactose unit using a sulfur trioxide/pyridine complex to provide compound **76**, which is then deblocked using conventional techniques, e.g., hydrogenolysis, to afford 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4-chloro-4-deoxy-3-O-sulfo-β-D-galactopyranosyl)]-β-D-glucopyranoside (**77**), after conversion to its sodium salt.

Figure 9 illustrates the synthesis of 4',6'-dichloro- and 4',6'-dideoxy-3'-sulfo-Lewis^{x}-OR derivatives. Specifically, in Figure 9, 3-O-benzyl-D-glucopyranose (**78**), prepared according to the procedure described by Finan, et al.[18], is converted into the 4,6-O-benzylidene derivative compound **79** using conventional procedures, e.g., reaction with benzaldehyde dimethylacetal (dimethoxytoluene) in the presence of an acidic catalyst, such as *p*-toluenesulfonic acid. Acetylation of compound **79**, preferably using acetic anhydride in pyridine, followed by removal of the benzylidene blocking group using 80% aqueous acetic acid then provides compound **80**, having unblocked hydroxyl groups at the 4 and 6 position of the glucose unit.

The unblocked hydroxyl groups of compound **80** are next converted to chloro substituents by treatment of compound **80** with a suitable chlorinating agent to provide acetyl 2-O-acetyl-3-O-benzyl-4,6-dichloro-4,6-dideoxy-glucopyranose (**81**). Preferably, compound **80** is contacted with sulfuryl chloride in a suitable solvent, such as pyridine. This reaction is typically conducted at a temperature in the range from about -40°C to about 20°C, preferably at -40°C, using an excess of sulfuryl chloride.

The benzyl blocking group of compound **81** is then removed under conventional hydrogenolysis conditions and the resulting unblocked 3-hydroxyl group is acylated using acetic anhydride in pyridine to provide compound **82**.

Compound **82** is next converted in two steps into α-(2,3-di-O-acetyl-4,6-dichloro-4,6-dideoxy-α-D-glucopyranosyl)-trichloroacetimidate (**83**). First, the anomeric acetate group of compound **82** is selectively removed by reaction with hydrazine acetate. This reaction is preferably conducted by contacting compound **82** with from about 1.1 to about 1.5 equivalents of hydrazine acetate (prepared by known procedures from hydrazine and

acetic anhydride) at a temperature of from about 0°C to about 20°C for about 5 to about 10 hours. Typically, the reaction is conducted in an anhydrous solvent, such as dimethylformamide. Treatment of the resulting product with trichloroacetonitrile in the presence of an amine, such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), provides compound **83**. This reaction is typically conducted in an anhydrous inert organic solvent, such as dichloromethane, using an excess of trichloroacetonitrile.

Compound **83** is then coupled with 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**56**), prepared according to the procedures described by Srivastava et al.[23], using conventional coupling conditions to provide 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)4-O-[2,3-di-O-acetyl-4,6-dichloro-4,6-dideoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**84**). The coupling reaction is generally conducted by contacting compound **56** with from about 1.1 to about 2 equivalents of trichloroacetimidate **83** and an excess of boron trifluoride etherate relative to the imidate. Preferably from about 1.1 to about 2 equivalents of boron trifluoride etherate are employed in this reaction. The reaction is typically conducted at from about -30°C to about 10°C (preferably -10°C) in a suitable anhydrous organic solvent such as dichloromethane or a 1:2 mixture of dichloromethane:ether.

Compound **84** is then deacetylated using convention conditions, e.g. sodium methoxide in methanol, to afford 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-dichloro-4,6-dideoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**85**)

Compound **85** is then sulfonated selectively on the 3-hydroxyl group of the galactose unit using a sulfur trioxide/pyridine complex to provide

compound **86**, which is then deblocked using conventional techniques, e.g., hydrogenolysis, to afford 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dichloro-4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl)]-β-D-glucopyranoside (**87**), after conversion to its sodium salt.

The 4′,6′-dideoxy-3′-sulfo-Lewis^x-OR derivative, compound **91**, can be prepared from the corresponding blocked 4′,6′-dichloro derivative, compound **84**, by reduction (e.g. de-chlorination) with tributyltin hydride. This reaction is typically conducted by contacting compound **84** with about 1.0 to about 10 equivalents of tributyltin hydride, preferably about 10 equivalents, in the presence of a free radical initiator, such as AIBN. The reaction is generally conducted in an inert solvent, such as toluene, at a temperature of from about 20° to about 80°C for a period of about 1 to about 7 hours. Reduction of compound **84** under these conditions affords compound **88**.

Compound **88** is then deacetylated, sulfonated and deblocked using conventional techniques to provide 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl)]-β-D-glucopyranoside (**91**), after conversion to its sodium salt.

Figure 10 illustrates the synthesis of 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4-deoxy-3-O-Sulfo-β-D-galactopyranosyl-2-deoxy-β-D-glucopyranoside sodium salt (**95**) from the blocked trisaccharide **74** described above. Specifically, reduction of the 4′-chloro group of compound **95** to the corresponding deoxy derivative is accomplished under conventional conditions using tributyltinhydride and azobisisobutyronitrile to provide for deoxy compound **92**. Deacetylation of the acetyl groups at the 2′ and 3′ positions followed by selective sulfonation with sulfur dioxide·pyridine complex leads to the 3′-sulfo

derivative, compound **94**. Subsequent removal of the remaining blocking groups via conventional methods leads to the title compound **95**.

H. 1-Azido Compounds and Derivatives Thereof

Figure 11 illustrates a reaction scheme for the synthesis of 1-azido-3'-sulfo-Lewis^{X} compounds. Subsequent reduction of the azido group leads to the amino group which, in turn, leads to amino derivatives of the formula 3'-sulfo-Lewis^{X}-NHR compounds where R is as defined above.

Specifically, in Figure 11, the known compound 3,4,6-triacetyl-2-acetamido-2-deoxy-α-D-glucopyranoside chloride **100** is converted to the 4,6-O-benzylidine-2-acetamido-2-deoxy-D-glucopyranoside azide, compound **103**, by conventional conditions well known in the art and described by Unverzagt, et al.[35] Specifically, compound **100**, as described by Srivastava, et al.[23], is first converted to the azido derivative by reaction with sodium azide in a suitable inert diluent such as dichloromethane:water in the presence of the phase transfer catalyst tri-*n*-capryl-methyl ammonium chloride (Aliquat 336) to provide for compound **101** which, in turn, is deacetylated by conventional Zemplin conditions (NaOMe/MeOH) and then treated under conventional conditions with *p*-methoxybenzyl dimethylacetal to provide for the 4,6-O-*p*-methoxybenzylidine derivative **103**.

The 4,6-O-*p*-methoxybenzylidine derivative, compound **103**, is then coupled with *p*-chlorophenyl 2,3,4-tri-O-*p*-methoxybenzyl-β-L-thiofucopyranoside, compound **104**, under conventional coupling conditions to provide for disaccharide **105**. Compound **104** is prepared in a manner disclosed in Example 1 of Srivastava, et al.[23] wherein the benzyl groups used in that example are replaced with *p*-methoxybenzyl groups.

The 4,6-O-benzylidene group of compound **105** is then opened regioselectively to provide for compound **106** having an unblocked hydroxyl group at th 4-position.

Subsequently, disaccharide **106** is converted to trisaccharide **108** by conventional coupling of O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-trichloroacetimidate, compound **107**, and deacetylation under conventional Zeplin conditions. The resulting compound is then selectively protected at the 4,6-positions by forming the 4,6-*p*-methoxybenzylidene derivative by reaction with *p*-methoxybenzaldehyde dimethylacetal in the presence of an acidic catalyst, such as pTSA, to provide for compound **109**. Selective sulfonation, as described above, provides for compound **110** which is followed by conventional removal of the protecting groups to provide for 1-azido-3'-sulfo-Lewis^{x} compound **111**.

Compound **111** provides for a convenient route for the synthesis of other compounds. Specifically, the azido group of compound **111** can be reduced by conventional means (e.g., hydrogenation with palladium on carbon) to provide for the amino derivative which can then be acetylated, alkylated, etc. by conventional means to provide for -NHR derivatives as illustrated in Examples 21-27 below. Conventional amine reactions well known in the art permit a wide range of -NHR compounds. If desired, the reactions of the amino group can be conducted while the reactive hydroxyl groups on the trisaccharide are blocked with removable blocking groups to avoid unintended reactions at these positions.

I. Deoxy/Sulfo Compounds

Figures 12 and 16 illustrate the synthesis of Lewis^{X}-YR compounds having a sulfo group on the galactose unit and further having one of the hydroxyl groups on the fucose unit being replaced with hydrogen.

The initial synthetic scheme provided in Figure 12 illustrates the preparation of deoxyfucose compounds which can be incorporated into the Lewis^{X} structures. Specifically, Figure 12 illustrates the literature conversion of methyl 2-*O*-benzyl-L-fucopyranoside, compound **201**, to methyl 2,3-di-*O*-benzyl-L-fucopyranoside, compound **202**, as reported by Deter-Jusynski, et al.[36] The free hydroxy in compound **202** is then converted to the corresponding methylthiothiocarbonyl derivative **203** by reaction carbon disulfide and methyl iodide or the corresponding imidazolethiocarbonyl derivative **204** by reaction with 1,1'-thio-carbonyldiimidazole. Reduction of either compound **203** or **204** provides for the corresponding deoxy derivative **205** which is then demethylated under acidic conditions to provide for 2,3-di-*O*-benzyl-4-deoxy-L-fucopyranose, compound **206**. In turn, compound **206** is treated with *p*-chlorothiophenol to provide for the corresponding *p*-chlorophenyl thiofucopyranoside, compound **207**, which is now ready for coupling to the N-acetylglucosamine unit.

Figure 12 illustrates one example of using compound **207** to couple with an N-acetylglucosamine unit. Specifically, compound **207** is coupled via conventional coupling conditions to disaccharide **208** disclosed in Internatinal Patent Application Serial No. PCT/US93/04909 to provide for fully protected trisaccharide **209**. The benzoyl groups of compound **209** are differentially removed with sodium hydroxide to provide for trisaccharide diol **210**. Selective sulfonation, as described above, provides for compound **211** which is followed by conventional removal of the protecting groups to provide for 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galacto-pyranosyl)-β-D-gluco-pyranoside, compound **212**.

Figure 16 illustrates another method for the preparation of Lewis^{X} analogues wherein the -YR algycon is -NHR. Specifically, the synthesis of

Figure 16 starts with compound **246** which was prepared in a manner similar to compound **103** with the exception that the N-acetyl group is replaced by N-phthalimido group and the azido group has been reduced to the amine and then converted to the -NHBz group by conventional methods. Compound **246** is then coupled to compound **207** to provide for disaccharide **247**. The benzylidene group of disaccharide **247** is then ring opened to provide for monohydroxy derivative **248** which is coupled to monosaccharide **249** to provide for trisaccharide **250**. Removal of the acetyl groups on the galactose unit of trisaccharide **250** provides for compound **251** which is then converted to the corresponding 4,6-di-*O*-benzylidene structure (compound **252**). Conventional removal of the N-phthalimido group provides for amine **253** which is then converted to the corresponding N-benzamido derivative **254**. Selective sulfonation, as described above, provides for compound **255** which is followed by conventional removal of the protecting groups to provide for compound **256**.

J. 3′,4″- and 3′,3″-Disulfo-Lewis^X Compounds

Figure 13 illustrates the synthesis of 3′,4″-disulfo-Lewis^X compounds. Specifically, in this figure, known *p*-chlorophenyl β-L-thiofucopyranoside, **213**, is converted to the corresponding protected 4,6-di-O-benzylidine structure, compound **214**, and then the hydroxyl group at the 2-position is blocked with a benzyl group to provide for compound **215**. Removal of the benzylidene group and selective benzylation of the 3-hydroxyl group leads to compound **217** which is then blocked at the remaining hydroxyl group with a *p*-methoxybenzyl group to provide for compound **218**.

Figure 13 also illustrates one example of using compound **218** to couple with an N-acetylglucosamine unit. Specifically, compound **218** is coupled via conventional coupling conditions to disaccharide **208** to provide

for fully protected trisaccharide **219**. The benzoyl groups of compound **219** are differentially removed with sodium hydroxide to provide for trisaccharide diol **220**. Selective sulfonation, as described above, provides for compound **221** which is followed by conventional removal of the *p*-methoxybenzyl group to provide for diol **222**. Again selective sulfation of compound **222** as described above leads to compound **223** followed by removal of the protecting groups to provide for 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-O-(4-O-sulfo-α-L-fucopyranosyl)-4-O-(3-O-sulfo-β-D-galacto-pyranosyl)-β-D-glucopyranoside, compound **224**.

Figure 14 illustrates the synthesis of 3',3"-disulfo-Lewis^{x} compounds such as 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(3-*O*-sulfo-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside, compound **232**.

Specifically, in this figure, *p*-chlorophenyl 2-*O*-benzyl-β-L-thiofucopyranoside, compound **216** (as described above), is converted to the corresponding protected 3-*O*-*p*-methoxybenzyl derivative, compound **225**, and then the hydroxyl group at the 4-position is blocked with a benzyl group to provide for compound **226**.

Figure 14 also illustrates one example of using compound **226** to couple with an N-acetylglucosamine unit. Specifically, compound **226** is coupled via conventional coupling conditions to disaccharide **208** to provide for fully protected trisaccharide **227**. The benzoyl groups of compound **227** are differentially removed with sodium hydroxide to provide for trisaccharide diol **228**. Selective sulfonation, as described above, provides for compound **229**. Selective removal of the *p*-methoxybenzyl protecting group on the 3-position of the fucose provides for compound **230** which is then selectively sulfonated, again as described above, to provide for compound **231**. Conventional removal of the remaining protecting groups

provides for 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(3-*O*-sulfo-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside, compound **232**.

K. 3′-sulfo-3″-methoxy-Lewis^{X} derivatives

Figure 15 illustrates the synthesis of 3′-sulfo-3″-methoxy-Lewis^{X} compounds such as 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(3-*O*-methyl-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside, compound **245**. Specifically, in this figure, known methyl 2-*O*-acetyl-L-fucopyranoside **233** (Zehavi, et al., J. Org. Chem., 37:13 (1977)) is converted to the corresponding protected 3-*p*-methoxybenzyl derivative, compound **234**. The acetyl group at the 2 position is removed and the 2,4-hydroxyl groups are blocked with benzyl groups to provide for compound **236**. Selective removal of the *p*-methoxybenzyl group at the 3-position provides for compound **237** which is then converted to the methylthiothiocarbonyl derivative, compound **238**. Reduction of this compound provides for the methoxy derivative, compound **239** which is then treated under acidic conditions to provide for the reducing sugar (**240**) which is converted under conventional conditions to the corresponding *p*-chlorophenyl thiofucopyranoside (compound **241**).

Figure 15 also illustrates one example of using compound **241** to couple with an N-acetylglucosamine unit. Specifically, compound **241** is coupled via conventional coupling conditions to disaccharide **208** to provide for fully protected trisaccharide **242**. The benzoyl groups of compound **242** are differentially removed with sodium hydroxide to provide for trisaccharide diol **243**. Selective sulfonation, as described above, provides for compound **244** which is followed by conventional removal of the protecting groups to provide for 3′-sulfo-3″-methoxy-Lewis^{X} compounds such as 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(3-*O*-methyl-α-L-

fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside, compound **245**.

Additional compounds which can be prepared via similar methods are described in Examples 32-52 below the synthesis of which is illustrated in Figures 17-36.

Lewis^{A} Analogues

The preparation of oligosaccharide glycosides having a 1→3 linkage between the galactose unit (or derivatized galactose unit) and the N-acetylglucosamine unit (or derivatized N-acetylglucosamine unit) as found in formula II above can be prepared by the procedures set forth above using appropriately protected N-acetylglucosamine compounds as described by, for example, Ippolito et al.[32]

Utility

Without being limited to any theory, it is believed that the oligosaccharide glycosides disclosed herein affect the cell mediated immune response in a number of ways. Specifically, these compounds can inhibit the ability of the immune response to become educated about a specific antigen when the compound is administered simultaneously with the first exposure of the immune system to the antigen.

Also, the oligosaccharide glycosides disclosed herein can inhibit the secondary immune response to an antigen in a sensitized mammal when administered after second or later exposures of the immune system to the same antigen. Additionally, the oligosaccharide glycosides disclosed herein can induce tolerance to antigens when administered at the time of second or later exposures of the immune system to the antigen.

The suppression of the inflammatory component of the secondary immune response by the oligosaccharide glycosides disclosed herein requires administering such compounds after initiation of the mammal's secondary immune response but at or prior to one-half the period required for maximal antigen induced inflammation. This criticality is disclosed in allowed U.S. Patent Application No. 08/081,214 entitled "Time Dependent Administration of Oligosaccharide Glycosides Related to Blood Group Determinants Having a Type I or Type II Core Structure in Reducing Inflammation in a Sensitized Mammal Arising from Exposure to an Antigen" which application is incorporated herein by reference in its entirety.

In this embodiment, the oligosaccharide glycosides of this invention are preferably administered to the patient at least about 0.5 hours after antigen exposure, more preferably, at least about 1 to 10 hour after antigen exposure, and still more preferably, from about at least about 1 to 5 hours after antigen exposure.

Similarly, in cell-mediated inflammatory responses arising from injuries (e.g., adult respiratory distress injury (lung injury)), administration of an oligosaccharide glycoside of this invention is also conducted after initiation of the immune response to this injury but at or prior to one-half the period required for maximal inflammation.

The oligosaccharide glycosides disclosed herein are effective in suppressing cell-mediated immune responses including cell-mediated immune response to an antigen (e.g., the inflammatory component of a DTH response) as well as in suppressing cell-mediated inflammatory responses to injury (e.g., lung injury) when administered at a dosage range of from about 0.5 mg to about 50 mg/kg of body weight per day, and preferably from about 0.5 to about 5 mg/kg of body weight per day. The

specific dose employed is regulated by the particular cell-mediated immune response being treated as well as by the judgment of the attending clinician depending upon factors such as the severity of the adverse immune response, the age and general condition of the patient, and the like. The oligosaccharide glycosides of this invention are generally administered parenterally, such as intranasally, intrapulmonarily, transdermally and intravenously, although other forms of administration are contemplated.

In addition to providing suppression of a mammal's secondary immune response to an antigen, administration of the oligosaccharide disclosed herein also imparts a tolerance to later challenges from the same antigen provided that the compound is administered during the critical period discussed above. In this regard, re-challenge by the same antigen weeks after administration of the oligosaccharide glycoside results in a significantly reduced immune response.

Administration of the oligosaccharide glycosides disclosed herein simultaneously with first exposure to an antigen (i.e., a non-sensitized mammal) imparts suppression of a cell-mediated immune response to the antigen and tolerance to future challenges with that antigen. In this regard the term "reducing sensitization" means that the oligosaccharide glycoside, when administered to a mammal in an effective amount along with a sufficient amount of antigen to induce an immune response, reduces the ability of the immune system of the mammal to become educated and thus sensitized to the antigen administered at the same time as the oligosaccharide glycoside. An "effective amount" of this compound is that amount which will reduce sensitization (immunological education) of a mammal, including humans, to an antigen administered simultaneously as determined by a reduction in a cell-mediated response to the antigen such as DTH responses as tested by the footpad challenge test. Preferably the reduction in sensitization will be at least about 20% and more preferably at

least about 30% or more. Generally, the oligosaccharide glycosides disclosed herein are effective in reducing sensitization when administered at a dosage range of from about 0.5 mg to about 50 mg/kg of body weight per day, and preferably from about 0.5 mg to about 5 mg/kg of body weight per day. The specific dose employed is regulated by the sensitization being treated as well as the judgement of the attending clinician depending upon the age and general condition of the patient and the like. "Simultaneous" administration of the compound with the antigen with regard to inhibiting sensitization means that the compound is administered once or continuously throughout a period of time within 3 hours of the administration of an antigen, more preferably the compound is administered within 1 hour of the antigen.

The methods of this invention are generally achieved by use of a pharmaceutical composition suitable for use in the parenteral administration of an effective amount of a oligosaccharide glycoside of this invention. These compositions comprise a pharmaceutically inert carrier such as water, buffered saline, etc. and an effective amount of a oligosaccharide glycoside so as to provide the above-noted dosage of these compounds when administered to a patient. Preferably, the pharmaceutical compositions comprise from about 1 to 99 weight percent of said inert carrier and from 99 to 1 weight percent of the oligosaccharide glycoside(s) described herein. It is contemplated that suitable pharmaceutical compositions can additionally contain optional components such as a preservative, etc.

It is also contemplated that other suitable pharmaceutical compositions can include oral compositions, transdermal compositions or bandages etc., which are well known in the art. Therefore, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups,

aerosols, ointments, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders. It is still further contemplated that the oligosaccharide glycoside compound can be incorporated as a part of a liposome or a micelle which can then be formulated into a pharmaceutical composition.

It is also contemplated that mixtures of these compounds can be used.

EXAMPLES

The following examples are set forth to illustrate the claimed invention and are not to be construed as a limitation thereof.

Examples 1-52 illustrate synthetic procedures for representative oligosaccharide glycosides of the present invention. Example A illustrates the suppression of antigen-induced inflammation in a mammal by administering the oligosaccharide glycoside compounds of Examples 7, 8, 9, 11, 12 and 13.

In these examples, the following abbreviations have the following meanings. If not defined, any abbreviation used in this application has its generally accepted meaning.

Ac	=	acetyl
AIBN	=	azobisisobutyronitrile
aq.	=	aqueous
Bn	=	benzyl
Bz	=	benzoyl
CAN	=	ceric ammonium nitrate
°C	=	degrees Celsius
d	=	doublet
DBU	=	1,8-diazabicyclo[5.4.0]undec-7-ene
DMF	=	dimethylformamide
DTH	=	delayed type hypersensitivity
EDTA	=	ethylene diamine tetraacetic acid
eq.	=	equivalents

g	=	gram
Gr	=	$-(CH_2)_8COCH_3$
^{1}H-nmr	=	proton nuclear magnetic resonance
kg	=	kilogram
L	=	liter
m	=	multiplet
M	=	molar
mg	=	milligrams
mL	=	milliliter
mmol	=	millimolar
MP	=	*p*-methoxy
N	=	normal
PMB	=	*p*-methoxybenzyl
s	=	singlet
THF	=	tetrahydrofuran
tlc	=	thin-layer chromatography
TR	=	trityl
μg	=	microgram
μL	=	microliter

Example 1 -- Synthesis of 8-Methoxycarbonyloctyl-2-benzamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 22)

The synthesis of compound **22** is illustrated in Figure 1.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-azido-2-deoxy-β-D-glucopyranoside (compound 1a)

3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide (synthesized according to the procedure described by Paulsen, et al.[19] and BeMiller, et al.[20]) (5 g, 12.6 mmol) in dichloromethane (5 mL) was added dropwise over a period of 0.5 hours to a stirred mixture of 8-methoxycarbonyloctanol (5.0 g), molecular sieves 4A (7.5 g, crushed) and dry silver carbonate (4.5 g) in dichloromethane (5 mL) at -20°C. The mixture was warmed to -10°C and stirred for 3-4 hours at which time analysis of the mixture by tlc (hexane-ethyl acetate; 60:40) indicated that the reaction was complete. The mixture was then diluted with dichloromethane and filtered on celite which had been washed twice with water. The solution

was then evaporated and the resulting crude product was dissolved in pyridine (30 mL) and acetylated with acetic anhydride (1.5 mL) at 22°C for 48 hours. Methanol was added to the mixture which was then diluted with dichloromethane, washed with water, a solution of sodium bicarbonate, water and brine. The crude product was chromatographed on silica gel, eluting with hexane-ethyl acetate (75:25), to give 8-methoxycarbonyloctyl-2-azido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (compound 1″) (5.5 g, 87%). The product was crystallized from ethanol. $[\alpha]_D^{22}$ -13.2° (C1.0, chloroform); m.p. 59-61°C; ^{1}H-nmr ($CDCl_3$) δ = 5.00(m, 2H, H-3 and H-4), 4.39 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 3.45-4.45 [m incl. OCH_3 (s, 3.67)], 2.10, 2.05 (2s, 6H, 2 OAc); Anal. Calc. for $C_{22}H_{35}O_{10}N_3$: C, 52.68; H, 7.03; N, 8.38, Found: C, 52.74; H, 6.90: N, 8.42.

A 0.2N solution of sodium methoxide in methanol (0.5 mL) was syringed into a flask containing the triacetate compound 1″ (5.5 g, 11.0 mmol) in methanol (160 mL). After 1 day at 22°C, Dowex(H^+) resin was added to the solution and the mixture was stirred, filtered and evaporated to give 8-methoxycarbonyloctyl-2-azido-2-deoxy-β-D-glucopyranoside (compound 1a) which was used directly in the next step without characterization.

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-2-azido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (compound 1)

Compound 1a (7.72 g, 20.6 mmol) from step (A) was dissolved in anhydrous acetonitrile (80 mL). Benzaldehyde dimethylacetyl (6.2 mL, 41.1 mmol) and a catalytic amount of *p*-toluenesulfonic acid (pTSA) were added and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was then neutralized with triethylamine and evaporated. The resulting syrup was chromatographed on silica gel using

hexane-ethyl acetate (4:1) as the eluent to provide for compound 1 (7.0 g, 73%).

Step (C) -- Preparation of 8-Methoxycarbonyloctyl-2-azido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (compound 3)

To a suspension of cupric bromide (12.31 g, 55.12 mmol) and molecular sieves (11.0 g) in dry dichloromethane (20 mL) under nitrogen were added DMF (8.5 mL, 110.3 mmol) and tetraethylammonium bromide (2.32 g, 11.03 mmol). After stirring the dark green mixture for approximately 30 minutes at room temperature, 8-methoxycarbonyloctyl-2-azido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (compound 1 -- 5.11 g, 11.03 mmol) was added. After stirring for 5 minutes, *p*-chlorophenyl tri-O-benzyl-fucose thioglycoside (compound 2 -- 11.92 g, 21.2 mmol), was added and the mixture was stirred for 15 hours at room temperature. The reaction mixture was then diluted with dichloromethane (500 mL) and filtered. The filtrate was washed with 5% EDTA solution (5 x 500 mL) and water (2 x 500 mL) and evaporated to give a syrup. Chromatography of the syrup on Iatrobeads, eluting with hexane-ethyl acetate (5:1), provided compound 3 (5.41 g, 55%).

Step (D) -- Preparation of 8-Methoxycarbonyloctyl-2-azido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 4)

Blocked disaccharide (compound 3 -- 5.0 g, 5.68 mmol), was dissolved in dry THF (20 mL) at room temperature. The reaction mixture was cooled and molecular sieves were added (500 mg, 3A). Sodium cyanoborohydride (5.27 g, 83.9 mmol) was then added along with a small amount of methyl orange (a few crystals). The reaction mixture was stirred for 15 minutes at 0°C and then an etheral solution of hydrochloric acid was added until the mixture was acidic (pH ~ 3). The reaction

mixture was stirred for 1 hours at 0°C at which time all the starting material had been completely consumed and converted to the product. The reaction mixture was then diluted with dichloromethane (500 mL) and triethylamine was added to neutralize (pH ~ 6 - 7) the mixture. The resulting mixture was filtered and the precipitate was washed thoroughly with dichloromethane (500 mL). The combined filtrates were then washed sequentially with a saturated solution of sodium bicarbonate (5 x 1L) and water (2 x 1L), and then evaporated to provide for compound 4 (3 g, 60%) as a syrup.

Step (E) -- Preparation of 8-Methoxycarbonyloctyl-2-azido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl]-6-O-benzyl--2-deoxy-β-D-glucopyranoside (compound 6)

Disaccharide acceptor (compound **4** -- 3.39 g, 3.84 mmol), and imidate donor (compound **5** -- 5.6 g, 11.35 mmol) were dissolved in a mixture of diethyl ether and dichloromethane (30 mL, 2:1) and stirred at -10°C for 15 minutes. Boron trifluoride etherate (2.33 mL, 18.92 mmol) was added and the reaction mixture was stirred under nitrogen for 15 hours at 0°C. The reaction mixture was then diluted with dichloromethane (500 mL), filtered and washed successively with a saturated solution of sodium bicarbonate (2 x 500 mL) and water (2 x 500 mL). Evaporation of the solvent gave a syrup which was purified by chromatography on Iatrobeads, eluting with hexane-ethyl acetate, to provide for compound **6** (3.5 g, 75%).

Step (F) -- Preparation of 8-Methoxycarbonyloctyl-2-azido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 7)

Trisaccharide **6** (4.81 g, 3.97 mmol) was dissolved in methanol (50 mL) and a catalytic amount of sodium methoxide (0.5 M solution) in methanol was added. The reaction mixture was stirred for 5 hours at room temperature and then neutralized with IR-120(H^+) ion exchange resin and

filtered. The filtrate was then evaporated and the resulting residue was purified by chromatography on silica gel, eluting with hexane-ethyl acetate (1:4), to provide tetrol trisaccharide **7** (4.0 g, 96.5 %).

Step (G) -- **Preparation of 8-Methoxycarbonyloctyl-2-azido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4,O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 8)**

To a solution of tetrol trisaccharide **7** (1.81 g, 1.73 mmol) dissolved in anhydrous acetonitrile (20.0 mL) was added benzaldehyde dimethylacetate (520.3 μL, 3.47 mmol) and a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture, which had a pH of ~3, was monitored by tlc. After stirring the reaction mixture for 5 hours at room temperature, tlc indicated that the starting material had been completely consumed and new spot having a higher Rf was observed. The reaction mixture was then neutralized with triethylamine (1.0 mL) and evaporated. The resulting residue was chromatographed on silica gel, eluting with hexane-ethyl acetate (1:2), to provide diol **8** (1.5 g, 77%).

Step (H) -- **Preparation of 8-Methoxycarbonyloctyl-2-amino-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 9)**

A solution of diol **8** (1.5 g, 1.32 mmol) in a mixture of pyridine, triethylamine and water (2:0.5:0.05, 20 mL) was saturated with a stream of hydrogen sulfide, initially at 0°C for 2 hours and then at room temperature for 15 hours. After stirring the reaction mixture for 15 hours at room temperature, the solvents were evaporated and then co-evaporated with toluene under high vacuum to remove pyridine. The resulting material was chromatographed on silica gel, eluting with hexane-ethyl acetate (1:2) and (1:4), to provide compound **9** (1.4 g, 96%).

Step (I) -- Preparation of 8-Methoxycarbonyloctyl-2-benzamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 11).

To a solution of amino sugar **9** (5.59 g, 5.05 mmol) in methanol (50 mL) was added a saturated sodium hydrogen carbonate solution (100 mL). The reaction mixture was then stirred at 0°C for 10 minutes. Benzoyl chloride (1.15 mL, 9.94 mmol) was then added and stirring was continued for 1 hour at room temperature by which time the starting material had been completely consumed. The reaction mixture was then diluted with dichloromethane (500 mL), filtered and evaporated. The residue was chromatographed on an Iatrobeads column, eluting with hexane-ethyl acetate (2:1), to provide compound **11** (5.0 g, 82%).

Step (J) -- Preparation of 8-Methoxycarbonyloctyl-2-benzamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 15)

To a solution of diol **11** (370 mg, 0.31 mmol) in pyridine (5.0 mL) at 0°C was added SO_3-pyridine complex (97.3 mg, 0.62 mmol) and the resulting reaction mixture was allowed to warm at room temperature. After stirring for 1 hour, tlc indicated that the starting material was not completely consumed so additional SO_3-pyridine complex (1 eq) was added and stirring was continued for 2 hours. At this time, tlc indicated that there was no remaining starting material. The mixture was then quenched with methanol (5.0 mL) and evaporated to dryness. The residue was chromatographed on Iatrobeads, eluting with dichloromethane-methanol-pyridine (95:5:0.5), to provided compound **15** (310 mg, 76%).

Step (K) -- Preparation of 8-Methoxycarbonyloctyl-2-benzamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 22).

A mixture of compound 15 (330 mg, 0.25 mmol) and 5% palladium on carbon (330 mg) in methanol (5.0 mL) was stirred under one atmosphere of hydrogen for 5 hours. The mixture was then filtered through a pad of celite to remove the catalyst and the catalyst/celite was washed with methanol (100 mL). The filtrate and washings were combined and evaporated after adding a trace of pyridine (5.0 mL). The resulting product was chromatographed on Iatrobeads, eluting with isopropanol-water-ammonia (7:15:0.5), to provide compound 22 (150 mg, 70%) after conversion to the sodium salt by passage through Dowex-50-x-8-(Na^+) resin.

Example 2 -- Synthesis of 8-Methoxycarbonyloctyl-2-*p*-nitrobenzamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 23)

The synthesis of compound 23 is illustrated in Figure 1.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-*p*-nitrobenzamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 12)

To a solution of amino sugar 9 (310 mg, 0.28 mmol) (prepared as described in Example 1) in methanol (4.0 mL) was added a saturated solution of sodium bicarbonate (1.0 mL). The reaction mixture was stirred for 15 minutes at room temperature, cooled to 0°C, and then *p*-nitrobenzoyl chloride (104 mg, 0.56 mmol) was added. After 15 minutes, tlc indicated that 75% of starting material had been consumed. Additional 4-nitrobenzoyl chloride (150.0 mg) was added and the reaction

mixture was stirred at room temperature for 15 hours. At this time, tlc indicated the reaction was essentially complete. The mixture was then diluted with dichloromethane (100 mL) and washed with a saturated solution of sodium bicarbonate (2 x 100 mL) and water (2 x 100 mL). Evaporation of the solvent gave a residue which was purified by chromatography on Iatrobeads, eluting with hexane-ethyl acetate (1:2), to provide compound **12** (250 mg, 71%).

Step (B) -- **Preparation of 8-Methoxycarbonyloctyl-2-*p*-nitrobenzamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-6-O-benzyl-β-D-glucopyranoside sodium salt (compound 16)**

To a solution of diol **12** (277 mg, 0.22 mmol) in pyridine (3.0 mmol) at 0°C was added SO_3-pyridine complex (52.7 mg, 0.33 mmol). The reaction mixture was stirred for 0.5 hours at 0°C and then for 2 hours at room temperature. An additional amount of SO_3-pyridine complex (1 eq) was then added and stirring was continued for 2 hours at room temperature. The excess reagent was destroyed by adding methanol and the mixture was evaporated. The resulting syrup was purified by chromatography on Iatrobeads, eluting with dichloromethane-methanol-pyridine (95:5:0.5), to provide compound **16** (250 mg, 84%).

Step (C) -- **Preparation of 8-Methoxycarbonyloctyl-2-*p*-nitrobenzamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 23)**

Compound **16** (350 mg, 0.26 mmol) was hydrogenolyzed in the presence of 5% palladium on carbon (350 mg) using the procedure described in Example 1, Step K for compound **15**. Conversion of the resulting product to its sodium salt by passage through Dowex-50-x-8 (Na^+) resin provided compound **23** (180 mg, 76%) after lyophilization.

Example 3 -- **Synthesis of 8-Methoxycarbonyloctyl-2-ortho-acetylbenzamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 24)**

The synthesis of compound 24 is illustrated in Figure 1.

Step (A) -- **Preparation of 8-Methoxycarbonyloctyl-2-ortho-acetylbenzamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 13)**

To a solution of amino sugar **9** (270 mg, 0.24 mmol) (prepared as described in Example 1) in dichloromethane (5.0 mL) at room temperature was added 2-*o*-acetylbenzoic acid (80 mg, 0.49 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (145 mg, 0.49 mmol). The reaction mixture was then stirred at room temperature overnight. Tlc indicated that some starting material remained so 1 additional equivalent of both reagents were added and stirring was continued for 15 hours. The reaction mixture was then diluted with dichloromethane, washed with water, dried over anhydrous sodium sulfate and evaporated. Chromatography of the residue on Iatrobeads, eluting with toluene-ethanol (95:5), provided compound **13** (250 mg, 83%).

Step (B) -- **Preparation of 8-Methoxycarbonyloctyl-2-ortho-acetylbenzamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 18).**

To a solution of diol **13** (300 mg, 0.24 mmol) in pyridine (3.0 mL) at 0°C was added SO_3-pyridine complex (75.2 mg, 0.47 mmol). The reaction mixture was stirred at 0°C for 1 hour and then at room temperature for 2 hours. Additional SO_3-pyridine complex (0.5 eq) was then added and stirring was continued for an additional 3 hours at room temperature. The excess SO_3-pyridine complex was destroyed by adding

methanol and the solution was evaporated. The residue was chromatographed on a silica gel, eluting with dichloromethane-methanol-pyridine (95:5:05), to provide compound **18** (250 mg, 77%) after conversion to a sodium salt by passage through Dowex 50-X-8 (Na^+) resin.

Step (C) -- Preparation of 8-Methoxycarbonyloctyl-2-ortho-acetylbenzamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 24)

Compound **18** (300 mg, 0.22 mmol) was hydrogenolyzed in the presence of 5% palladium on carbon (300 mg) using the procedure described in Example 1, Step K for compound **15**. Conversion of the resulting product to its sodium salt by passage through Dowex-50-x-8 (Na^+) resin provided compound **24** (150 mg, 75%) after lyophilization.

Example 4 -- Synthesis of 8-Methoxycarbonyloctyl-2-cyclohexamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 25)

The synthesis of compound **25** is illustrated in Figure 1.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-cyclohexamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 14)

To a solution of amino sugar **9** (270 mg, 0.24 mmol) (prepared as described in Example 1) in dichloromethane (50 mL) at room temperature was added cyclohexanecarboxylic acid (31.27 mg, 0.49 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimidate hydrochloride (145 mg, 0.49 mmol). The reaction mixture was stirred for 15 hours at room temperature and then an additional 0.49 mmol amount of each reagent was added and stirring was continued overnight. The mixture was then diluted

with dichloromethane (100 mL) and the dichloromethane layer washed with water (2 x 100 mL), dried and evaporated. The residue was chromatographed on Iatrobeads, eluting with dichloromethane-methanol (97:3), to provided compound **14** (230 mg, 79%).

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-2-cyclohexamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 17)

To a solution of compound **14** (231.5 mg, 0.19 mmol) in dry pyridine (3.0 mL) at 0°C was added SO_3-pyridine complex (45.5 mg, 0.29 mmol). The solution was allowed to warm at room temperature by which time tlc indicated that all the starting material had been consumed. The excess SO_3-pyridine complex was destroyed by adding methanol (1 mL) and the reaction mixture was evaporated. The resulting residue was chromatographed on an Iatrobeads column eluting with dichloromethane-methanol-pyridine (95:5:0.5), to provide compound **17** (225 mg, 90%) after conversion to a sodium salt by passage through Dowex 50-X-8 (Na^+) resin.

Step (C) -- Preparation of 8-Methoxycarbonyloctyl-2-cyclohexamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 25)

Compound **17** (216 mg, 0.16 mmol) was hydrogenolyzed in the presence of 5% palladium on carbon (200 mg) using the procedure described in Example 1, Step K for compound **15**. Conversion of the resulting product to its sodium salt by passage through Dowex-50-x-8 (Na^+) resin provided compound **25** (120 mg, 86%) after lyophilization.

Example 5 -- Synthesis of 8-Methoxycarbonyloctyl-2-fuc(C)amido-3-O-(α-L-fucopyranosyol)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 26)

The synthesis of compound **26** is illustrated in Figure 1.

Step (A) -- Preparation of C-Fucoside

To a stirred mixture of 1-allyl-1-deoxy-2,3,4-tri-O-acetyl-α-L-fucopyranose (10 g, 31.9 mmols) (prepared as described by Luengo, et al.[33]) in a solvent mixture of acetonitrile:carbon tetrachloride:water (80 mL:80 mL:120 mL)), was added 28.0 g (131.2 mmole) of sodium periodate followed by ruthenium trichloride hydrate (145 mg) in the manner described by Carlson et al.[34]. The reaction became exothermic after 10 minutes and was stirred overnight at room temperature. The mixture was diluted with water (300 mL) and extracted with dichloromethane (2 x 300 mL). The combined organic layer was washed with water (100 mL) and concentrated. The residual oil was dissolved in ethyl acetate (200 mL) and extracted with saturated sodium bicarbonate (30 mL). The organic layer was washed again with water (20 mL) which was combined with the sodium bicarbonate solution extracts. This combined aqueous extract was acidified with 6N hydrochloric acid solution to pH 1 and extracted with dichloromethane (2 x 200 mL). The combined organic extracts were washed with water (100 mL), followed by saturated sodium chloride solution, dried over sodium sulfate, filtered and concentrated to give 6.9 g (20.8 mmole, 65%) of (1-deoxy-2,3,4-tri-O-acetyl-α-L-fucopyranoside) acetic acid. Deacetylation of this compound provides for fucose(C)carboxylic acid.

If desired, the fucose(C)carboxyic acid can be converted to its NHS-C-fucoside via conventional methods. For example, (1-deoxy-2,3,4-tri-O-

acetyl-α-L-fucopyranoside) acetic acid (1.97 g, 6.16 mmol) was dissolved in dichloromethane (25 mL) and N-hydroxysuccinimide (NHS, 1.0 g, 8.69 mmol) was added to the solution, and the solution was warmed to dissolve the NHS. Dicyclohexylcarbodiimide (DCC, 1.41 g, 6.83 mmol) was dissolved in dichloromethane (5 mL) and added to the reaction mixture with stirring. After 5 hours, the reaction mixture was cooled to 4°C, filtered and evaporated. The syrupy residue was taken up in ethyl acetate (50 mL), filtered and washed with water (2 x 25 mL). The ethyl acetate layer was dried over anhydrous sodium sulfate, filtered and evaporated. After drying under high vacuum, 2.5 g (94%) of an amorphous white solid (1-deoxy-2,3,4-tri-O-acetyl-α-L-fucopyranosyl) acetic acid N-hydroxysuccinimide ester was obtained.

Step (B) -- **Preparation of 8-Methoxycarbonyloctyl-2-(2,3,4-tri-O-acetyl-fuc-(C)-amido)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 19)**

To a solution of amino sugar 9 (270 mg, 0.24 mmol) (prepared as described in Example 1) in dichloromethane (2.0 mL) at room temperature was added fucose(C)carboxylic acid (443 mg) and 1-(3-dimethylamino-propyl)-3-ethylcarbodiimidate hydrochloride (443 mg). The reaction mixture was stirred for 15 hours at room temperature and then diluted with dichloromethane (100 mL). The organic layer was washed with water (3 x 100 mL), dried over anhydrous sodium sulfate and evaporated. The residue was then chromatographed on Iatrobeads, eluting with dichloromethane-methanol (95:5), to provided compound 19 (286 mg, 84%).

Step (C) -- **Preparation of 8-Methoxycarbonyloctyl-2-(2,3,4-tri-O-acetyl-fuc(C)amido)-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 20)**

To a solution of diol **19** (286 mg, 0.2 mmol) in anhydrous dichloromethane (3.0 mL) at 0°C was added SO_3-pyridine complex (2 eq). The reaction mixture was allowed to warm to room temperature and was stirred for 3 hours. The excess SO_3-pyridine complex was destroyed by adding methanol (1.0 mL) and the solution was then evaporated. The resulting residue was chromatographed on an Iatrobead column, eluting with dichloromethane-methanol-pyridine (95:5:0.5), to provided compound **20** (250 mg, 82%) after conversion to the sodium salt by passage through Dowex 50-X-8 (Na^+) resin.

Step (D) -- Preparation of 8-Methoxycarbonyloctyl-2-fuc(C)amido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 21)

To a solution of compound **20** (240 mg, 0.158 mmol) in methanol (5.0 mL) was added sodium methoxide in methanol (0.5 M solution). The resulting solution was stirred for 5 hours at room temperature and then neutralized with IR-120(H^+) ion exchange resin, filtered (the filtered residue was washed with methanol) and evaporated. The resulting residue was chromatographed on Iatrobeads, eluting with dichloromethane-methanol-pyridine (95:5:0.5), to provide compound **21** (180 mg, 82%) after conversion to the sodium salt by passage through Dowex 50-X-8 (Na^+) resin.

Step (E) -- Preparation of 8-Methoxycarbonyloctyl-2-fuc(C)amido-3-O-(α-L-fucopyranosyol)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 26)

Compound **21** (250 mg, 0.179 mmol) was hydrogenolyzed in the presence of 5% palladium on carbon (250 mg) using the procedure described in Example 1, Step K for compound **15**. Conversion of the

resulting product to its sodium salt by passage through Dowex-50-x-8 (Na^+) resin provided compound **26** (150 mg, 88%) after lyophilization.

Example 6 -- Synthesis of 8-Methoxycarbonyloctyl-2-amino-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (27)

The synthesis of compound **27** is illustrated in Figure 1.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-azido-3-O-(3,4,6,-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 10)

To a solution of diol **8** (5.0 g, 4.42 mmol)(prepared as described in Example 1) dissolved in pyridine (50.0 mL) at 0°C was added SO_3-pyridine complex (1.05 g, 6.62 mmol). The reaction mixture was stirred for 0.5 hours at 0°C and then for 1 hour at room temperature. The reaction was not complete, so additional SO_3-pyridine complex (500 mg) was added and stirring was continued for an additional 2 hours. The reaction mixture was then quenched with methanol and the solvent evaporated. Chromatography of the resulting syrup using dichloromethane-methanol-pyridine (95:5:0.5) as eluent provided compound **10** (4.0 g, 73%) after sodium ion exchange column.

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-2-amino-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 27)

Compound **10** (250 mg, 0.2 mmol) was dissolved in methanol (5.0 mL) containing 0.1% HCl in MeOH and 5% palladium on carbon (250 mg) was added. The reaction mixture was stirred for 6 hours at room temperature and then the catalyst was removed by filtration. Pyridine was added and the solution was evaporated. Conversion of the resulting

product into its sodium salt by passage through Dowex-50-x-8 (Na^+) resin provided compound **27** (120 mg, 78%) as a white powder after lyophilization.

Example 7 -- Synthesis of 8-Methoxycarbonyloctyl-2-*p*-nitrobenzamido-4-O-(β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranose (30)

The synthesis of compound **30** is illustrated in Figure 2.

8-methoxycarbonyloctyl-2-amino-4-O-(β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranose (**28**) (250 mg, 0.489 mmol), prepared by glycosylation of 2-azido-2-deoxy-3,6,2',3',4',6'-hexa-O-acetyl-α-D-lactopyranosyl bromide[22] with 8-methoxycarbonyloctanol followed by reduction with H_2S in pyridine:triethylamine:water (4:1:0.1) and deacetylation, was added to pyridine (5 mL) followed by addition of *p*-nitrobenzoyl chloride (2.5 g). The reaction mixture was stirred for 15 hours at room temperature and then excess *p*-nitrobenzoyl chloride was destroyed by adding methanol (5 mL). The mixture was evaporated and the residue was diluted with dichloromethane (100 mL). This solution was washed with a 5% solution of hydrochloric acid (2 x 100 mL), aqueous sodium bicarbonate (2 x 100 mL) and water (2 x 100 mL), then dried over sodium sulfate, filtered and evaporated to dryness to give compound **29** which was used without further purification.

Compound **29** was debenzoylated by dissolving the compound in sodium methoxide (0.5 M solution) in methanol and stirring the resulting mixture for 15 hours at room temperature. The reaction mixture was then neutralized by adding IR-120(H+) ion exchange resin and the resin was removed by filtration. The filtrate was evaporated to dryness and the resulting residue was purified by chromatography on Iatrobeads, eluting with dichloromethane-methanol-water (80:20:2), to provide compound **30** (210 mg, 68% based on compound **28**).

Example 8 -- Synthesis of 8-methoxycarbonyloctyl-4-O-(4-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside sodium salt (compound 38)

The synthesis of compound **38** is illustrated in Figure 3.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-4-O-(2,3,6-tri-O-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (compound 36)

To a solution of 8-methoxycarbonyloctyl lactopyranoside (compound **31**) (9.5 g, 18.5 mmol) (prepared as described in Banoub, et al.[24]) in acetonitrile (100 mL) at room temperature was added 2,2-dimethoxypropane (4.56 mL, 37 mmol) and *p*-toluenesulfonic acid (95 mg). After stirring for 3 hours at room temperature, the reaction mixture was neutralized by adding trimethylamine (1.0 mL). The mixture was then evaporated to dryness and the residue filtered through silica gel using dichloromethane-methanol (80:20) to provide the 3,4- and 4,6-O-isopropylidene derivatives **32** and **33**.

The mixture of compounds **32** and **33** prepared above was dissolved in pyridine and dichloromethane (50 mL) (1:9) and cooled to 0°C. Benzoyl chloride (10.0 mL) was added dropwise at 0°C and resulting mixture was stirred for 15 hours at room temperature. The excess benzoyl chloride was then neutralized by adding methanol (5.0 mL) and the reaction mixture evaporated. The residue was diluted with dichloromethane (250 mL), washed with 5% aq. hydrochloric acid (2 x 250 mL), saturated sodium bicarbonate (2 x 250 mL) and water (2 x 250 mL), dried over anhydrous sodium sulfate, filtered and evaporated. The resulting residue was chromatographed on silica gel, eluting with dichloromethane-methanol (98:2) and (95:5), to provide a mixture of compounds **34** and **35** (14.5 g).

The mixture of compounds **34** and **35** prepared above was dissolved in 80% aqueous acetic acid and this solution was stirred at room temperature for 15 hours. The mixture was then evaporated and co-evaporated with toluene to dryness. The resulting residue was then benzoylated by adding benzoyl chloride (1.3 eq) in a mixture of dichloromethane-pyridine (9:1) at -50°C. Standard work-up procedures gave a residue (one major spot by tlc) which was chromatographed on silica gel, eluting with hexane-ethyl acetate (2:1), to provide compound **36** (11.5 g).

Step (B) – Preparation of 8-Methoxycarbonyloctyl-4-O-(4-O-sulfo-2,3,6-tri-O-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside sodium salt (compound 37)

To a solution of compound **36** (50 mg, 0.44 mmol) in pyridine (2.0 mL) was added SO_3-pyridine complex (14 mg, 0.88 mmol). The resulting mixture was stirred for 1 hour at room temperature. Additional SO_3-pyridine complex (36 mg) was then added and stirring was continued for 3 hours. The reaction mixture was quenched with methanol (1.0 mL) and evaporated to dryness. The residue was chromatographed on silica gel, eluting with dichloromethane-methanol-pyridine (95:5:0.5), to provide compound **37** (50 mg, 92%) after conversion to sodium salt by passage through Dowex-50-x-8 (Na^+) resin.

Step (C) – Preparation of 8-Methoxycarbonyloctyl-4-O-(4-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside sodium salt (compound 38)

To a solution of compound **37** (50 mg, 0.04 mmol) in methanol (5.0 mL) was added a 0.5 molar solution of sodium methoxide in methanol. The resulting solution was stirred at room temperature for 15 hours. The mixture was then neutralized by adding IR-120 (H^+) resin, filtered and evaporated to dryness. The residue was chromatographed on silica gel,

eluting with dichloromethane-methanol-water-pyridine (80:20:2:2), to provide compound **38** (20.0 mg, 81%) after sodium exchange and lyophilization.

Example 9 -- Synthesis of 8-Methoxycarbonyloctyl-4-O-(4-O-phospho-β-D-galactopyranosyl)-β-D-glucopyranoside disodium salt (compound 40)

The synthesis of compound **40** is illustrated in Figure 3.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-4-O-(4-O-phospho-2,3,6-tri-O-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside disodium salt (compound 39)

To a solution of compound **36** (1.0 g, 0.88 mmol) in dry pyridine (5.0 mL) at 0°C was added dimethylaminopyridine (161 mg, 1.32 mmol) and diphenylphosphorochloride (365 μL, 1.76 mmol). The resulting mixture was stirred at 0°C for 1 hour and then at room temperature for 5 hours by which time tlc showed complete consumption of the starting material. The mixture was then diluted with dichloromethane (250 mL), washed with cold 5% HCl solution (2 x 250 mL), saturated sodium bicarbonate solution (2 x 250 mL) and water (2 x 250 mL), and evaporated to dryness. The residue was chromatographed on a silica gel, eluting with dichloromethane-methanol (95:5), to provided compound **39** (1.0 g, 83%).

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-4-O-(4-O-phospho-β-D-galactopyranosyl)-β-D-glucopyranoside disodium salt (compound 40)

A solution of compound **39** (750 mg, 0.55 mmol) in methanol (20.0 mL) containing PtO_2 on carbon (750 mg) was hydrogenolysed for 15 hours at 1 atmosphere of hydrogen. The catalyst was filtered and the solution was evaporated after adding pyridine (1.0 mL). The residue was saponified by reacting with sodium methoxide (0.5 molar solution) in methanol using standard conditions and workup. The debenzoylated

product was chromatographed on an Iatrobeads column, eluting with isopropanol-water-ammonia (7:3:1), to provide compound **40** (300 mg, 86%).

Example 10 -- Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(3,6-di-O-sulfo-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside disodium salt (compound 43)

The synthesis of compound **43** is illustrated in Figure 4.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(β-D-galactoypyranosyl)-2-deoxy-4,6-O-benzylidene-β-D-glucopyranoside (compound 41)

8-Methoxycarbonyloctyl-2-acetamido-3-O-(β-D-galactoypyranosyl)-2-deoxy-4,6-O-benzylidene-β-D-glucopyranoside, compound **41**, was prepared by coupling with thiobenzyl-2,3,4,6-tetraacetyl-β-D-galactopyranoside (reported by Ippolito et al.[32]) with the 8-methoxycarbonyloctyl-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (also reported by Ippolito et al.[32]). Specifically, 1.5 equivalents of *n*-iodosuccinimide, 1 equivalent of 8-methoxycarbonyloctyl-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (glycosyl donor), 1.5 equivalents of thiobenzyl-2,3,4,6-tetraacetyl-β-D-galactopyranoside (glycosyl acceptor) and molecular sieves were first combined in dichloromethane and then stirring the mixture for 0.5 hours at -20°C. Then, 1 to 1.5 equivalents of trifluoromethane sulfonic acid was added and the reaction mixture stirred for 3 hours at -20°C. The reaction mixture was quenched by adding triethyamine until neutral pH was reached. The reaction solution was filtered, washed with sodium bicarbonate and water. The organic layer was dried over sodium sulfate and the solvent evaporated to provide for the fully protected 8-methoxycarbonyloctyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside which was then

subjected to zemplen conditions (sodium methoxide/methanol) to provide for 8-methoxycarbonyloctyl-2-acetamido-3-O-(β-D-galactoypyranosyl)-2-deoxy-4,6-O-benzylidene-β-D-glucopyranoside, compound **41**.

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(3,6-di-O-sulfo-β-D-galctopyranosyl)-2-deoxy-4,6-O-benzylidene-β-D-glucopyranoside disodium salt (compound 42)

To a solution of 8-methoxycarbonyloctyl-2-acetamido-3-O-(β-D-galactopyranosyl)-2-deoxy-4,6-O-benzylidene-β-D-glucopyranoside (compound 41) (1.5 g, 2.34 mmol) (prepared as described above) in pyridine (7.5 mL) at 0°C was added SO_3-pyridine complex (4.0 equivalent). The reaction mixture was stirred for 5 hours at room temperature, quenched by adding methanol (2 mL), and evaporated to dryness. The residue was chromatographed on Iatrobeads, eluting with dichloromethane-methanol-pyridine (9:1:0.5), to provide compound 42 (700 mg, 35%) after conversion to a sodium salt using the procedures described earlier.

Step (C) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(3,6-di-O-sulfo-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside disodium salt (compound 43)

Compound 42 (550 mg, 0.65 mmol) was dissolved in methanol (50 mL) and hydrogenolysed in the presence of 5% palladium on carbon (550 mg) using the procedure described previously. The mixture was filtered, evaporated and chromatographed on an Iatrobeads column, eluted with dichloromethane-methanol-water-pyridine (80:20:2:2), to provide compound **43** (350 mg, 71%) after conversion to the sodium salt as described above.

Example 11 -- Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(3,6-diphospho-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside tetrasodium salt (compound 45)

The synthesis of compound **45** is illustrated in Figure 4.

To a solution of compound **41** (1.2 g, 1.87 mmol) (prepared as described above) in pyridine (10.0 mL) at 0°C was added dimethylaminopyridine (1.5 eq) and diphenylphosphorochloride (4.0 eq). The resulting mixture was stirred at 0°C for 5 hours and then diluted with dichloromethane (100 mL), washed with cold 5% HCl solution (2 x 100 mL), saturated sodium bicarbonate solution (2 x 100 mL) and water (2 x 100 mL), dried over sodium sulfate, filtered and evaporated to dryness. The residue was chromatographed on silica gel, eluting with dichloromethane-methanol (95:5), to provide compound **44** (850 mg, 41%).

Compound **44** (750 mg, 0.68 mmol) was hydrogenolysed in the presence of PtO_2 on carbon (750 mg) in methanol using standard procedures and work-up conditions to provide deblocked phospho-Le^c, compound **45** (400 mg, 73%).

Example 12 -- Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3,4,6-tri-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside trisodium salt (compound 48)

The synthesis of compound **48** is illustrated in Figure 5.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[3,4,6-tri-O-sulfo-β-D-galactopyranosyl]-2-deoxy-6-O-benzyl-β-glucopyranoside trisodium salt (compound 47)

To a solution of 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**46**) (2.5 g, 2.36 mmol) (prepared as described by Srivastava, et al.[23]) in pyridine (15.0 mL) at 0°C was added SO_3-

pyridine complex (5 eq). This reaction mixture was stirred at 0°C for 0.5 hours and then overnight at room temperature. The mixture was then quenched with methanol (5.0 mL), evaporated and the residue chromatographed on Iatrobeads using dichloromethane-methanol-pyridine (9:1:0.5) and dichloromethane-methanol-water-pyridine (80:20:2:0.5) as an eluent to provide compound 47 (1.0 g, 31%) as a syrup.

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3,4,6-tri-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside trisodium salt (compound 48)

Compound **47** (800 mg, 0.59 mmol) was hydrogenolysed with palladium on carbon (800 mg) using the procedures and work-up conditions as described above to provide compound **48** (400 mg, 67%) as the sodium salt.

Example 13 -- Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 52)

The synthesis of compound **52** is illustrated in Figure 6.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(3,4,6-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[3-benzoyl-4,6-O-benzylidene-2-O-(3,4,6-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 49)

Compound **49** was prepared by selective benzoylation at the 3′ of 8-methoxycarbonyloctyl-2-acetamido-3-O-(3,4,6-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (prepared as described by Srivastava[31]) followed by fucosylation at the 2′ position using $CuBr_2$/DMF catalyzed

reaction conditions and tribenzyl thiofucose as the glycosyl donor in methylene chloride.

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(3,4,6-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-2-O-(3,4,6-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 50)

To a solution of 8-methoxycarbonyloctyl-2-acetamido-3-O-(3,4,6,-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[3-O-benzoyl-4,6-O-benzylidene-2-O-(3,4,6-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound **49**) (1.45 g, 0.87 mmol) (prepared by as above) (1.45 g, 0.87 mmol) in dichloromethane was added sodium methoxide (0.5 M solution) in methanol and the resulting solution was stirred for 5 hours at room temperature. The mixture was then neutralized with resin, filtered and evaporated to dryness to obtain compound **50** (1.2 g, 88%) which was used without further purification.

Step (C) -- Preparation of 8-Methoxycarbonyloctyl-3-O-(3,4,6-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-2-O-(3,4,6-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 51)

To a solution of compound **50** (150 mg, 0.096 mmol) in pyridine (2.0 mL) at 0°C was added SO_3-pyridine complex (30.5 mg, 0.19 mmol). The resulting mixture was stirred for 20 minutes at 0°C and then overnight at room temperature. Standard work-up procedures as described above gave a residue which was chromatographed on Iatrobeads, eluting with dichloromethane-methanol-pyridine (95:5:0.5), to provide compound **51** (135 mg, 84%).

Step (D) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[3-O-sulfo-2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 52)

Blocked sulfo-tetrasaccharide **51** (120 mg, 0.072 mmol) was hydrogenolyzed over palladium on carbon (120 mg) using the procedures and work-up conditions described above to provide for compound **52** (54 mg, 79%).

Example 14 -- Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[6-chloro-6-deoxy-3-O-sulfo-β-D-galactopyranosyl)]-β-D-glucopyranoside sodium salt (compound 60)

The synthesis of compound **60** is illustrated in Figure 7.

Step (A) -- Preparation of 6-Chloro-6-deoxy-1,2,3,4-tetra-O-acetyl-glucose (compound 54)

1,2,3,4-Di-O-isopropylidene-D-galactopyranose (compound **53**) (11.33 g, 43.6 mmol) was dissolved in 200 mL of dry pyridine and cooled to -40°C. Sulfuryl chloride (11 mL) was added and the reaction mixture was stirred at -40°C for 1 hour. The reaction was then warmed to room temperature and stirring was continued for 3 hours at room temperature. Dichloromethane (1 L) was added and the resulting solution was washed with a saturated solution of sodium bicarbonate and twice with water. The solvent was evaporated and the residue was chromatographed on a silica gel column using hexane-ethyl acetate (5:1) as the eluent. The isolated product was dissolved in 200 mL of 90% trifluoroacetic acid and the resulting mixture was stirred at room temperature for 1 hour and then evaporated and co-evaporation with toluene. The residue was then acetylated in a mixture of pyridine-acetic anhydride (1:1, 300 mL) for 20 hrs. After standard work-up conditions, the residue was chromatographed, eluting

with hexane-ethyl acetate (3:1), to provide tetra-O-acetyl-6-chloro-6-deoxy-D-galactopyranose (compound **54**) (6.36 g, 40%) as a foam.

Step (B) -- Preparation of O-(2,3,4-Tri-O-acetyl-6-Chloro-6-deoxy-α-D-glucopyranosyl) Trichloroacetimidate (compound 55)

To a solution of tetra-O-acetyl-6-chloro-6-deoxy-D-galactopyranose (compound **54**) (6.36 g, 17.3 mmol) in DMF (60 mL) was added hydrazine acetate (2.4 g, 26.0 mmol). The mixture was stirred for 1 hour at room temperature and then 1 L of dichloromethane was added and the resulting solution washed 4 times with water. The organic layer was dried over anhydrous sodium sulfate and evaporated. Flash column chromatography using hexane-ethyl acetate (1:1) as the eluent gave 2,3,4-tri-O-acetyl-6-chloro-6-deoxy-α-D-galactopyranose (5.79 g). This material was dissolved in 50 mL of dichloromethane and cooled to 0°C. Trichloroacetonitrile (8.93 mL, 89.2 mmol) was added followed by DBU (1.33 mL, 8.9 mmol). This solution was stirred for 1.5 hours and then evaporated. The residue was chromatographed on a silica gel column, eluting with hexane-ethyl acetate (2:1), to provide compound **55** (4.1 g, 51%) as a foam.

Step (C) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranoysl)-4-O-[6-chloro-6-deoxy-2,3,4-tri-O-acetyl-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 57)

To a solution of compound **56** (9.7 g, 10.8 mmol) (prepared by the procedure described by Srivastava, et al.[23]) in 35 mL of dichloromethane-diethyl ether (1:2), stirred under nitrogen, was added imidate **55** (10.3 g 21.9 mmol) and then an additional 10 mL of dichloromethane-ether (1:2). The resulting mixture was cooled to -10°C to -15°C at which temperature a gel type mass formed. Dichloromethane was added until the solution became clear and then 3.5 mL of BF_3-ethereate solution was added dropwise and stirring was continued for 1 hour at low temperature. The

reaction mixture was then diluted with dichloromethane and washed with water, saturated $NaHCO_3$ solution and water. The resulting solution was dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on a silica gel column, eluting with hexane-ethyl acetate (1:1), to provide compound 57 (12.09 g, 93%) as a white foam.

Step (D) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-chloro-6-deoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 58)

To a solution of compound 57 (5 g, 4.12 mmol) in 160 mL of methanol was added 10 mL of a 0.5 N solution of sodium methoxide in methanol. The resulting mixture was stirred at room temperature overnight and then neutralized with Amberlite IR-120 (H^+) resin, filtered and evaporated. The residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (97:3), to provide compound **58** (3.93 g, 87%).

Step (E) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-chloro-6-deoxy-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 59)

To a solution of compound **58** (3.92, 3.63 mmol) in pyridine (50 mL) at 0°C was added SO_3-pyridine complex (860 mg, 1.5 eq). The resulting mixture was stirred at 0°C for 0.5 hours and then allowed to warm to room temperature while stirring. After 0.5 hours at room temperature, 0.5 eq. of SO_3-pyridine complex was added. An additional 0.5 eq. of SO_3-pyridine complex was added after 1.5 hours. After 3 hours, the reaction was terminated by adding methanol. The solvent was evaporated and the resulting residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol-pyridine (95:5:05). After

evaporation, the product was dissolved in methanol and passed through Bio-Rex® 70 resin (100 - 200 mesh, sodium form) using methanol as an eluent to provide compound **59** (2.82 g, (66%).

Step (F) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[6-chloro-6-deoxy-3-O-sulfo-β-D-galactopyranosyl)]-β-D-glucopyranoside sodium salt (compound 60)

To a solution of compound **59** (537 mg, 0.46 mmol) in methanol (50 mL) was added (530 mg) of 5% Pd/C. The resulting mixture was hydrogenolyzed at room temperature for 2.5 hours at atmospheric pressure and then filtered and evaporated. The residue was chromatographed on an Iatrobead column, eluting with dichloromethane-methanol-water-pyridine (80:20:2:0.5). The fractions were pooled and evaporated and passed through Bio-Rex® 70 Resin (100-200 mesh, sodium form) using methanol as an eluent. Freeze-drying gave compound **60** (285 mg, 76%) as a white solid.

Example 15 -- Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[6-deoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 63)

The synthesis of compound **63** is illustrated in Figure 7.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4,-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-deoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 61)

A mixture of compound **57** (6.1 g, 5.1 mmol), tributyltinhydride (13.6 mL, 10 eq) and AIBN (115 mg) in toluene (250 mL) was heated at 90°C for 4 hours. The reaction mixture was then evaporated and the residue was chromatographed on a silica gel column, eluting with hexane-

ethyl acetate (1:1), to provide crude compound **60a** as a foam (~6.2 g with some impurities and tin residue). This crude material was dissolved in a mixture of methanol (100 mL) and dichloromethane (20 mL) and 10 mL of a 0.5 N solution of sodium methoxide in methanol was added. The reaction mixture was stirred overnight at room temperature and neutralized with Amberlite IR-120(H^+) resin, filtered and evaporated. The residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (95:5), to provide compound **61** (4.42 g, 83%) as a white foam.

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-0-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-deoxy-3-O-sulfo-β-D-galactopyranosyl]-6-benzamido-2-deoxy-β-D-glucopyranoside sodium salt (compound 62)

To a solution of compound **61** (4.42 g, 4.2 mmol) in 50 mL of pyridine at 0°C was added SO_3-pyridine complex (1 g , 1.5 eq). The reaction mixture was stirred for 0.5 hours at 0°C and then allowed to warm to room temperature. After 0.5 hours at room temperature, 0.5 eq. of SO_3-pyridine complex was added followed by an additional 0.5 eq. after 1.5 hours. After 3 hours, the reaction was terminated by adding methanol and the solvents were evaporated. The residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (95:5) containing 2 mL of pyridine per 1 L of solvent, and the resulting product was passed through Bio-Rex® 70 Resin (100-200 mesh, sodium form) using methanol as an eluent to provide compound **62** (2.65, 55%).

Step (C) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)4-O-[6-deoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 63)

A mixture of compound **62** (562 mg, 0.49 mmol) and 560 mg of 5% Pd/C in 50 mL of methanol was hydrogenolyzed for 2 hours under standard conditions. The mixture was then filtered, evaporated and

chromatographed on an Iatrobead column, eluting with dichloromethane-methanol-water (80:20:2) containing pyridine (2 mL per 1 L of eluent), to provide a product which was then passed through Bio-Rex® 70 Resin (100-200 mesh, sodium form) using water as an eluent. The resulting solution was freeze-dried to provide compound 63 (216 mg, 56%).

Example 16 -- Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4-chloro-4-deoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 77)

The synthesis of compound 77 is illustrated in Figure 8.

Step (A) -- Preparation of Methyl 4-6-O-Benzylidene-2,3-di-O-benzoyl-α-D-glucopyranoside (compound 65)

To a mixture of 25 g (0.13 mmol) of methyl-α-D-glucopyranoside (64) (commercially available from Aldrich Chemical Co., Milwaukee, Wisconsin, USA) in 750 mL of acetonitrile was added α,α-dimethoxytoluene (20 mL) and *p*-toluenesulfonic acid (600 mg). The resulting mixture was stirred at room temperature for 20 hours and then neutralized with triethylamine and evaporated. The residue was dissolved in pyridine (200 mL) and cooled in an ice bath. Benzoyl chloride (5 eq) was then added and the mixture was stirred overnight at room temperature. After standard work-up procedures, compound 65 (44.2 g, 70%) was crystallized from a mixture of ethyl acetate and ether.

Step (B) -- Preparation of Methyl 6-O-Benzyl-2,3-di-O-benzoyl-α-D-glucopyranoside (compound 66)

A mixture of methyl 4,6-O-benzylidene-2,3-di-O-benzoyl-α-D-glucopyranoside (compound 65) (16.3 g, 33.2 mmol), sodium cyanoborohydride (42.9 g, 0.68 mmol), 3A molecular sieves and crystals of methyl orange in 500 mL of tetrahydrofuran was stirred at 0°C while diethyl ether saturated with hydrogen chloride was added until the solution

became pink and evolution of gas ceased. After 1 hour, tlc indicated that the starting material had been consumed. The reaction mixture was then poured into a cold mixture of dichloromethane and a saturated aqueous solution of sodium bicarbonate. The organic layer was separated and washed with saturated sodium bicarbonate solution and water (5 times), then dried over anhydrous sodium sulfate and evaporated to provide crude methyl 6-O-benzyl-2,3-di-O-benzoyl-α-D-glucopyranoside (compound **66**) as a white solid which was used in the next step without further purification.

Step (C) -- Preparation of Methyl 6-O-Benzyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-α-D-glucopyranoside (compound 67)

To a solution of crude methyl 6-O-benzyl-2,3-di-O-benzoyl-α-D-glycopyranoside (compound **66**) (from previous step) in 300 mL of pyridine at -40°C was added sulfuryl chloride (15 mL). After 1 hour, the cooling bath was removed and stirring was continued at room temperature for 3 hours. Dichloromethane was then added and the resulting solution was washed with saturated sodium bicarbonate solution and water, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on a silica gel, eluting with dichloromethane, to provide methyl 6-O-benzyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-α-D-galactopyranoside (compound **67**) (15.3 g, 90.0% -- based on compound **65**) as a syrup.

Step (D) -- Preparation of Acetyl 6-O-acetyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-D-glucopyranoside (compound 68)

To a solution of methyl 6-O-benzyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-α-D-galactopyranoside (compound **67**) (14.4 g, 28.2 mmol) in 75 mL of acetic anhydride was added a mixture of acetic anhydride and concentrated sulfuric acid (60 mL, 13 μL of conc. H_2SO_4 per 1 mL of acetic anhydride). The resulting mixture was stirred at room temperature

overnight and then the solvent was evaporated under high vacuum. The residue was dissolved in dichloromethane and this solution was washed with saturated sodium bicarbonate solution and water, and evaporated. The residue was chromatographed on a silica gel column, eluting with hexane-ethyl acetate (3:1), to provide acetyl 6-O-acetyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-D-galactopyranoside (compound **68**) (8.53 g, 62%).

Step (E) -- Preparation of α-(6-O-acetyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-α-D-galactopyranosyl)-trichloroacetimidate (compound 69)

A solution of acetyl 6-O-acetyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-D-galactopyranoside (**68**) (8.53 g, 17.4 mmol) and hydrazine acetate (2.4 g, 26.0 mmol, 1.5 eq) in 70 mL of DMF was stirred for 1 hour at room temperature. The reaction mixture was then evaporated, washed with water (5 times) and evaporated. The residue was chromatographed on a silica gel, eluting with hexane-ethyl acetate (2:1), to provide 6-O-acetyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-D-galactopyranose (7.44 g, 95.3%). This material was dissolved in dichloromethane (50 mL) and the resulting solution was cooled to 0°C. Trichloroacetonitrile (8.3 mL, 82.9 mmol) was added and the mixture was stirred for 2 hours. The solvent was then evaporated and the residue was chromatographed on a silica gel column, eluting with hexane-ethyl acetate (3:1), to give imidate **69** (6.3 g, 64%).

Step (F) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-O-acetyl-2,3-di-O-benzoyl-4-chloro-4-deoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 70)

To a solution of compound **56** (1.98 g 2.2 mmol) (prepared by the procedure described by Srivastava, et al.[23]) in 10 mL of dichloromethane-ether (1:2) was added imidate **69** (2.45g, 4.13 mmol) and 4 mL of dichloromethane-diethyl ether (1:2). The reaction mixture was then cooled to -15°C to -10°C and BF_3-etherate (0.7 mL) was added. This mixture

was stirred for 1 hour and then diluted with dichloromethane. The resulting solution was washed with sodium bicarbonate solution and water, and then evaporated to give a syrup. The syrup was chromatographed on silica gel, eluting with hexane-ethyl acetate (2:1) and (1:1), to give trisaccharide **70** (1.42 g, 49%) as a white solid.

Step (G) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4-chloro-4-deoxy-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 71)

To a solution of compound **70** (1.42 g 1.1 mmol) in 20 mL of methanol was added 5 mL of 0.5 N sodium methoxide in methanol. The reaction mixture was stirred at room temperature for 5 hours and then neutralized with Amberlite IR-120 (H^+) resin, filtered and evaporated. The residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (95:5), to provide compound **71** (1.11g, 94%).

Step (H) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)4-O-[2,3-di-O-acetyl-4-chloro-4-deoxy-6-*tert*-butyl-dimethylsilyl-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 72)

A solution of compound **71** (1.05 g, 0.97 mmol) and *tert*-butydimethylsilyl chloride (296 mg, 1.96 mmol) in pyridine (10 mL) was stirred overnight at room temperature. Acetic anhydride (5 mL) was added at 0°C and stirring was continued for 3 hours at room temperature. The solution was worked-up followed by chromatography using hexane-ethyl acetate (1:1) as an eluent gave compound **72** (1.08 g, 87%).

Step (I) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[2,3-di-O-acetyl-4-chloro-4-deoxy-β-D-galactopyranosyl]-2-deoxy-6-O-benzyl-β-D-glucopyranoside (compound 73)

To a solution of compound 72 (1.07 g , 0.84 mmol) in 25 mL of THF was added tetrabutylammonium fluoride hydrate (399 mg 1.26 mmol). The resulting mixture was stirred at room temperature for 1 hour and then diluted with dichloromethane (100 mL), washed with water (2 x 100 mL) and evaporated. The residue was chromatographed, eluting with hexane-ethyl acetate (1:1), to provide compound 73 (815 mg, 84%).

Step (J) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[2,3-di-O-acetyl-6-O-benzyl-4-chloro-4-deoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 74)

To a solution of compound 73 (815 mg, 0.7 mmol) in 10 mL of toluene was added benzyl bromide (167 mL, 1.4 mmol) and silver oxide (715 mg, 3.5 mmol). The reaction mixture was stirred at room temperature for 24 hours and then filtered and evaporated. The residue was chromatographed on a silica gel column, eluting with hexane-ethyl acetate (1:1), to provide compound 74 (471 mg, 54%).

Step (K) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-O-benzyl-4-chloro-4-deoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 75)

To a solution of compound 74 (467 mg, 0.34 mmol) in 10 mL of methanol was added 5 mL of 0.5N sodium methoxide in methanol. The resulting mixture was stirred for 3 hours at room temperature and then neutralized with Amberlite IR-120(H^+) resin, filtered and evaporated. The

residue was chromatographed on a silica gel, eluting with dichloromethane-methanol (98:2), to provide compound **75** (390 mg, 90%).

Step (L) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[6-O-benzyl-4-chloro-4-deoxy-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 76)

To a solution of compound **75** (390 mg, 0.34 mmol) in pyridine (1 mL) at 0°C was added SO_3-Pyridine complex (80 mg, 0.5 mmol). The reaction mixture was allowed to warm to room temperature and additional SO_3-pyridine complex was added after 0.5 hours (0.5 eq) and 1 hour (0.5 eq). The reaction was terminated after 2 hour by adding methanol and the solvents were then evaporated. The residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (95:5) containing 2 mL of pyridine per 1 L of solvent, to provide compound **76** (241 mg, 57%) after Bio-Rex® 70 Resin (sodium form) ion exchange.

Step (M) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4-chloro-4-deoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 77)

A solution of compound **76** (241 mg, 0.19 mmol) in methanol (20 mL) containing 240 mg of 5% Pd/C was hydrogenolyzed at room temperature for 1.5 hours. The reaction mixture was then filtered and evaporated. The residue was chromatographed, eluting with dichloromethane-methanol (80:20:2) containing 2 mL of pyridine per 1 L of solvent, to provide a product which was passed through a Bio-Rex®70 Resin (sodium form) column using water as an eluent. The resulting solution was freeze-dried to give compound **77** (135 mg, 87%).

Example 17 – Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dichloro-4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-β-D-glucopyranoside sodium salt (compound 87)

The synthesis of compound 87 is illustrated in Figure 9.

Step (A) – Preparation of 3-O-Benzyl-4,6-O-benzylidene glucopyranose (compound 79)

A solution of 3-O-benzyl-D-glucopyranose (78) (12.8 g, 47.4 mmol) (prepared by the procedure described by Finan, et al.[18]), α,α-dimethoxytoluene (10 mL) and *p*-toluenesulfonic acid (265 mg) in 500 mL of acetonitrile was stirred at room temperature for 1.5 hours. The mixture was then neutralized with triethylamine and evaporated. The residue was chromatographed, eluting with dichloromethane-methanol, to provide compound 79 (5.21 g, 31%).

Step (B) – Preparation of Acetyl 2-O-acetyl-3-O-benzyl-D-glucopyranose (compound 80)

3-O-Benzyl-4,6-O-benzylidene-D-glucopyranose (compound 79) (5.21 g, 14.5 mmol) was acetylated in mixture of pyridine (40 mL) and acetic anhydride (140 mL) at room temperature for 2 hours. After standard work-up procedures and evaporation of the solvent, the residue was heated with 80% aqueous acetic acid (500 mL) for 17 hours. Flash column chromatography using hexane-ethyl acetate (1:2) as an eluent gave acetyl 2-O-acetyl-3-O-benzyl-D-glucopyranoside (compound 80) (3.5 g, 68%).

Step (C) – Preparation of Acetyl 2-O-Acetyl-3-O-benzyl-4,6-dichloro-4,6-dideoxy-glucopyranose (compound 81)

To a solution of acetyl 2-O-acetyl-3-O-benzyl-D-glucopyranose (compound 80) (3.5 g, 9.88 mmol) in pyridine at -40°C was added a solution of SO_2Cl_2 (100mL) in 100 mL of pyridine. The reaction mixture

was allowed to warm to room temperature and was stirred for 3 hours. The mixture was then diluted with dichloromethane (500 mL) and this solution was washed with water (2 x 500 mL), saturated sodium bicarbonate solution and water (2 x 500 mL), dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on a silica gel column, eluting with hexane-ethyl acetate (5:1), to provide acetyl 2-O-acetyl-3-O-benzyl-4,6-dichloro-4,6-dideoxy-D-galactopyranoside (compound **81**) (2.89 g, 75%).

Step (D) -- Preparation of Acetyl-2,3-di-O-acetyl-4,6-dichloro-4,6-dideoxy-glucopyranose (compound 82)

A solution of acetyl-2-O-acetyl-3-O-benzyl-4,6-dichloro-4,6-dideoxy-D-galactopyranoside (compound **81**) (2.89 g, 7.4 mmol) in 60 mL of methanol was hydrogenolyzed in the presence of 1.4 g of 5% Pd/C for 3 hours. The solution was then filtered and evaporated and the residue was acetylated in a mixture of pyridine (10 mL) and acetic anhydride (10 mL). Standard work-up procedures and column chromatography using hexane-ethyl acetate (2:1) as an eluent provided acetyl 2,3-di-O-acetyl-4,6-dichloro-4,6-dideoxy-D-galactopyranose (compound **82**) (2.34 g, 92%).

Step (E) -- Preparation of α-(2,3-Di-O-acetyl-4,6-dichloro-4,6-dideoxy-α-D-glucopyranosyl)-trichloroacetimidate (compound 83)

A solution of acetyl 2,3-di-O-acetyl-4,6-dichloro-4,6-dideoxy-D-galactopyranose (compound **82**) (2.24 g, 6.5 mmol) and hydrazine acetate (903 mg) in DMF (22mL) was stirred for 2 hours at room temperature. The reaction mixture was then diluted with dichloromethane (250 mL) and washed 4 times with water (4 x 250 mL), dried over anhydrous sodium sulfate and evaporated. Flash chromatography using hexane-ethyl acetate (1:1) as an eluent gave 2,3-di-O-acetyl-4,6-dichloro-4,6-dideoxy-D-galactopyranose (~6.88 mmol). This compound was then dissolved in dichloromethane (20 mL), cooled to 0°C, and trichloroacetonitrile (3.35

mL, 34.4 mmol) was added. After stirring at 0°C for 40 minutes, the solvent was evaporated and the residue was chromatographed on a silica gel column, eluting with hexane-ethyl acetate (5:1), to give imidate **83** (1.14g, 39%) as a white solid.

Step (F) – Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[2,3-di-O-acetyl-4,6-dichloro-4,6-dideoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 84)

To a solution of compound **56** (990 mg, 1.10 mmol) in dichloromethane-diethyl ether (1:2) (5 mL), stirred under nitrogen, was added dichloro imidate **83** (997 mg, 2.94 mmol) followed by additional dichloromethane-diethyl ether (1:2) (2 mL). The solution was cooled to -10°C to -15°C over 1 hour, at which temperature a gel-type mass formed. Dichloromethane was added until solution became clear and then BF_3-etherate solution (350 μL) was added dropwise. The reaction mixture was stirred for 1 hour and then diluted with dichloromethane (250 mL), washed with $NaHCO_3$ solution (2 x 250 mL) and water (2 x 250 mL), dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on a silica gel, eluting with hexane-ethyl acetate (2:1) and (1:1), to provide compound **84** (853 mg, 66%).

Step (G) – Preparation of 8-Methyoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-dichloro-4,6-dideoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 85)

To a solution of compound **84** (402 mg, 0.34 mmol) in 20 mL of methanol was added 2 mL of 0.5N sodium methoxide in methanol. The resulting mixture was stirred at room temperature for 1 hour and then neutralized with Amberlite (IR-120 (H^+) resin, filtered, evaporated under high vacuum. The residue was chromatographed on a silica gel column,

eluting with dichloromethane-methanol (95:5), to provide compound **85** (358 mg, 96%).

Step (H) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-dichloro-4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (86)

To a solution of compound **85** (356 mg, 0.32 mmol) in pyridine (7 mL) at 0°C was added SO_3-pyridine complex (70 mg, 0.44 mmol). The reaction mixture was stirred for 30 minutes at 0°C then the cooling bath was removed and stirring was continued at room temperature. Additional SO_3-pyridine complex was added after 1 hour (0.5 eq), 2.5 hours (0.5 eq), 3.5 hours (0.5eq) and 4 hours (1.5 eq). The reaction was then terminated after 5.5 hours by adding methanol. The solvents were evaporated and the residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (95:5) containing 2 mL of pyridine per 1 L of solvent, to provide compound **86** (240 mg, 63%) after Bio-Rex® 70 Resin (sodium form).

Step (I) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dichloro-4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-β-D-glucopyranoside sodium salt (compound 87)

A solution of compound **86** (215 mg, 0.18 mmol) in 20 mL of methanol containing 215 mg of 5% Pd/C was hydrogenolyzed at room temperature and atmospheric pressure for 6 hours. The solution was then filtered and evaporated. The residue was chromatographed on an Iatrobead column using dichloromethane-methanol-water (80:20:2) containing pyridine (12 mL per 1 L of solvent) as eluent. The collected material was passed through Bio-Rex® 70 Resin (sodium form) using water as an eluent

and the resulting solution was freeze-dried to provide compound 87 (128 mg, 85%).

Example 18 -- Synthesis of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 91)

The synthesis of compound 91 is illustrated in Figure 9.

Step (A) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[2,3-di-O-acetyl-4,6-dideoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 88)

A mixture of blocked di-chloro trisaccharide (compound 84) (412 mg, 0.35 mmol), tributyltin hydride (1.88 mL, 6.98 mmol) and AIBN (20 mg) in 20 mL of toluene was heated at 90°C for 3 hours. The solvent was then evaporated and the residue was chromatographed on a silica gel column, eluting with hexane-ethyl acetate (2:1), to provide compound 88 (298 mg, 77%).

Step (B) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-dideoxy-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 89)

To a solution of compound 88 (298 mg, 0.27 mmol) in methanol (10 mL) and dichloromethane (2 mL) was added a 0.5 N solution of sodium methoxide in methanol (5 mL). After 2 hours at room temperature, the reaction mixture was neutralized with Amberlite IR-120(H^+) resin, filtered and evaporated. The residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (95:5), to provide compound 89 (253 mg, 91%).

Step (C) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (compound 90)

To a solution of compound **89** (253 mg, 0.23 mmol) in 5 mL of pyridine at 0°C was added SO_3-pyridine complex (59 mg, 0.37 mmol). After 0.5 hours at 0°C, the cooling bath was removed and stirring was continued at room temperature. Additional SO_3-pyridine complex was added after 1.5 hours (0.75 eq), 2.5 hours (0.75 eq) and 3.5 hours (0.75 eq). After 5 hours, the reaction was terminated by adding methanol. The solvents were evaporated and the residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (95:5) containing pyridine (2 mL per 1 L of solvent), to provide compound **90** (227 mg, 87%) after passing the recovered product through Bio-Rex® 20 Resin (sodium form) using methanol as an eluent.

Step (D) -- Preparation of 8-Methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4,6-dideoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (compound 91)

A solution of compound **90** (225 mg, 0.20 mmol) in 20 mL of methanol containing 225 mg of 5% Pd/C was hydrogenolyzed for 2 hours at room temperature. The solution was then filtered and evaporated. The residue was chromatographed on an Iatrobeads column, eluting with dichloromethane-methanol-water (80:20:2) containing 2 mL of pyridine per liter of solvent, to provide a product which was converted into its sodium salt by passage through Bio-Rex® 70 Resin (sodium form) using water as an eluent. The resulting solution was freeze-dried to give compound **91** (147 mg, 96%).

Example 19 -- Synthesis of 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4-deoxy-3-O-sulfo-β-D-galactopyranosyl-2-deoxy-β-D-glucopyranoside sodium salt (95)

The synthesis of compound **95** is illustrated in Figure 10.

Step (A) Preparation of 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[2,3-di-O-acetyl-4-deoxy-6-O-benzyl-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (compound 92)

A mixture of blocked trisaccharide (compound 74) (374 mg, 0.30 mmol), tributyltinhydride (1.7 mL) and AIBN (10 mg) in 20 mL of toluene was heated at 90°C for 3-5 hours. The solvent was evaporated and the residue was chromatographed on a silica gel column eluting with hexane-ethyl acetate (1:1) to provide for compound **92** (325 mg, 89%).

Step (B) Preparation of 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4-deoxy-6-O-benzyl-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside (93)

A 0.5 M solution of sodium methoxide in methanol (5.0 mL) was added to a soltuion of compound **92** (325 mg, 0.27 mmol) in methanol (10 mL). After stirring for 3 hours at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and the solvent evaporated. The residue was chromatographed on a silica gel column eluting with dichloromethane-methanol (97:3) to provide compound **93** (289 mg, 95.5%).

Step (C) Preparation of 8-methoxycarbonyloctyl-2-acetamido-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-4-O-[4-deoxy-3-O-sulfo-6-O-benzyl-β-D-galactopyranosyl]-6-O-benzyl-2-deoxy-β-D-glucopyranoside sodium salt (94)

To a solution of compound **93** (289 mg, 0.26 mmol) in 5 mL of pyridine at 0°C was added SO_3-pyridine complex (51 mg, 0.38 mmol) and the reaction mixture was stirred for 15 minutes at 0°C and then 2-5 hours at room temperature. Additional amounts of SO_3-pyridine complex were added (0.7 equivalents) after 1 and 2 hours. After 5.5 hours, the reaction was terminated by adding methanol. The solvents were evaporated and the residue was chromatographed on a silica gel column, eluting with dichloromethane-methanol (95:5) containing pyridine (2 mL per 1 L of solvent), to provide compound **94** (266 mg, 84.4%), after passing the recovered product through Bio-Rex® 70 Resin (sodium form) using methanol as eluent.

Step (D) Preparation of 8-methoxycarbonyloctyl-2-acetamido-3-O-(α-L-fucopyranosyl)-4-O-[4-deoxy-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside sodium salt (95)

A solution of compound **94** (266 mg, 0.22 mmol) in 10 mL of methanol containing 266 mg of 5% palladium on carbon was hydrogenolyzed for 2 hours at room temperature. The solution was then filtered and evaporated. The residue was chromatographed on an Iatrobead column, eluting with dichloromethane-methanol-water (80:20:2) containing a bit of pyridine (2 mL/1L of the solvent mixture) to provide a product which was converted into a sodium salt by passage through BioRex® 70 Resin (sodium form) using water as the eluent. The residue was freeze dried to give compound **95** (145 mg, 86.9%).

Example 20 -- Preparation of 2-Acetamido-2-deoxy-3-*O*-[α-L-fucopyranosyl]-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl azide sodium salt (compound 111)

The synthesis of compound **111** is illustrated in Figure 11.

Step (A) Preparation of 2-Acetamido-2-deoxy-3-*O*-[2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl]-4,6-*O*-*p*-methoxybenzylidine-β-D-glucopyranosyl azide (compound 105)

Copper (II) bromide (59.7 g, 0.268 mol, 5.0 eq.) and pulverized 4A molecular sieves (100 g) were added to a reaction vessel. Dry dichloromethane (120 mL) was syringed into the flask followed by dry DMF (47 mL, 10 eq.). Tetraethylammonium bromide (11.2 g, 0.0535 mol, 1.0 eq.) was added to the reaction flask and the greenish-black mixture was stirred for 30 minutes. The acceptor 2-*N*-acetamido-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-β-D-glucopyranosyl azide, compound **103** (19.5 g, 0.0535 mol, 1.0 eq.), was added to the reaction, followed by the donor *p*-chlorothiophenyl 2,3,4-tri-*O*-*p*-methoxybenzyl-β-L-fucopyranoside, compound **104** (43.5 g, 0.0668 mol, 1.25 eq.). The mixture was stirred for three hours at room temperature before being quenched with triethylamine until neutral (3 mL, pH=7). The reaction was filtered through celite and washed with 800 mL of dichloromethane. The organic layer was washed 4 x 1 L with a 5% EDTA solution (made by mixing 150 g tetrasodium EDTA salt with 75 g of fully protonated EDTA, pH=7). The organic layer was washed once with 1.5 L of water, dried ($MgSO_4$), filtered and concentrated to give a golden syrup. This syrup was recrystallized from ethyl acetate/hexane to give 33.1 g of compound **105** (71%). MW 870.97 ($C_{46}H_{54}O_{13}N_4$).

Step (B) Preparation of 2-Acetamido-2-deoxy-3-*O*-[2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl]-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide (compound 106)

The benzylidene acetal (compound **105** -- 23.2 g, 26.6 mmol, 1 eq.), sodium cyanoborohydride (16.7 g, 266 mmol, 10 eq.), 3A molecular

sieves (50 g), and methyl orange (2 mg) were charged to a round bottom flask. THF (350 mL) was poured into the flask and the mixture was cooled to 0°C. A solution of hydrochloric acid in ether was added in a dropwise fashion to the reaction mixture until a pink color persisted. After 3 hours, the reaction was quenched by pouring the mixture into 600 mL of saturated sodium bicarbonate solution. Dichloromethane (600 mL) was added and the organic layer was separated. The organic layer was washed with 3 x 500 mL of bicarbonate solution, 1 x 500 mL water and 1 x 500 mL of a saturated solution of sodium chloride. The organic layer was dried ($MgSO_4$), filtered and concentrated. The golden syrup was recrystallized from isopropanol to give 19 g (82%) of compound **106** as a white solid. MW 872.98 ($C_{46}H_{56}O_{13}N_4$).

Step (C) Preparation of 2-Acetamido-2-deoxy-3-*O*-[2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl]-4-*O*-[β-D-galactopyranosyl]-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide

The acceptor 2-Acetamido-2-deoxy-3-*O*-[2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl]-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide (compound **106** -- 15.0 g, 0.017 mol) and the donor 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl trichloroacetimidate (compound **107** -- 12.5 g, 0.025 mol, 1.5 eq.) were added to a round bottomed flask. Dichloromethane, 200 mL, was poured into the round bottomed flask and the mixture was cooled to 0°C. Boron trifluoride etherate (1.05 mL, 8.5 mmol, 0.5 eq.) was syringed into the mixture. The reaction continued for eight hours and was quenched with triethylamine (0.6 mL). The final pH of the reaction mixture was 7. The reaction was concentrated and purified by silica gel chromatography. The eluent used was a step gradient (25%-60% ethyl acetate in hexane). Compound **108** (15.5 g, 75%) was obtained as a yellowish foam. MW 1203.28 ($C_{60}H_{74}O_{22}N_4$).

The tetraacetylated galactose intermediate (compound **108** -- 15.5 g, 0.0129 mol) was dissolved in 200 mL of methanol. A sodium methoxide in methanol solution (5 mL of a 0.5 M solution, 2.5 mmol, 0.2 eq) was syringed into the mixture. The mixture was stirred overnight and neutralized with Amberlite IRC 120 H^+ resin. The neutralized mixture was filtered and concentrated. The product was purified by silica gel chromatography. The eluent used was 3-5% methanol in dichloromethane. The product (11.2 g, 83%) was obtained as a white solid. MW 1035.13 ($C_{52}H_{66}O_{22}N_4$).

Step (D) Preparation of 2-Acetamido-2-deoxy-6-*O*-*p*-methoxybenzyl-3-*O*-[2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl]-4-*O*-[4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl]-β-D-glucopyranosyl azide (compound 109)

2-Acetamido-2-deoxy-3-*O*-[2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl]-4-*O*-[β-D-galactopyranosyl]-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide (11.2 g, 10.8 mmol, 1 eq.) was dissolved in 150 mL of acetonitrile. Anisaldehyde dimethyl acetal (3.7 mL, 22 mmol, 2 eq.) was syringed into the stirred mixture. Toluene sulfonic acid (50 mg) was added to the reaction mixture ensuring a pH of 2-3. The reaction was stirred at pH 2-3 for 30 minutes at room temperature. The reaction was quenched by neutralization with triethylamine. The solution was concentrated and purified by silica gel chromatography. The chromatography eluent was 2% methanol in dichloromethane. The product, compound **109**, obtained was a white solid (10.2 g, 82%). MW 1153.26 ($C_{60}H_{72}O_{19}N_4$).

Step (E) Preparation of 2-Acetamido-2-deoxy-3-*O*-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl)-4-*O*-[4,6-*O*-*p*-methoxybenzylidene-3-*O*-sulfo-β-D-galactopyranosyl]-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide sodium salt (compound 110)

2-Acetamido-2-deoxy-3-O-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl)-4-*O*-[4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl]-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide (compound **109** -- 4.02 g, 3.49 mmol, 1 eq.) was dissolved in pyridine (40 mL) and cooled to 0°C. Sulfur trioxide pyridine complex (1.11 g, 6.97 mmol, 2 eq) was added to the reaction mixture. The reaction was slowly allowed to reach room temperature. After 90 minutes another portion of sulfur trioxide pyridine complex was added (0.28 g, 0.5 eq.). After a total reaction time of three hours, the reaction was quenched by the addition of 2 mL of methanol. The solution was concentrated and purified by silica gel chromatography. The chromatographic eluent was 5% methanol in dichloromethane with 0.5% added pyridine. After purification, the product was concentrated and passed through 50 g of analytical grade Na^+ ion exchange resin using methanol as the eluent. The product was concentrated to give compound **110** as a white solid (3.98 g, 92%). MW 1255.30 ($C_{60}H_{71}O_{22}N_4SNa$).

Step (F) Preparation of 2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl azide sodium salt (compound 111)

2-N-Acetamido-2-deoxy-3-O-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyransoyl)-4-*O*-[4,6-*O*-*p*-methoxybenzylidene-3-*O*-sulfo-β-D-galactopyranosyl]-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide sodium salt (1.0 g, 0.797 mmol) was dissolved in 30 mL of 90% aqueous acetonitrile. The reaction mixture was cooled to 0°C and ceric ammonium nitrate (3.5 g, 6.4 mmol, 8 eq.) was added to the mixture all at once. The reaction was gradually warmed to room temperature and quenched at 6.5 hours by the addition of pyridine. The reaction required 1.7 mL of pyridine for complete neutralization. The reaction was concentrated, and purified by column chromatography using Iatrobead silica gel. The chromatographic eluent was 70:30:3:1 dichloromethane:methanol:water:pyridine. The product was concentrated to dryness and passed through

40 g of analytical grade Na^+ ion exchange resin. The product was then filtered through a 0.22 μ millipore filter and lyophilized. The product, compound **111**, was isolated as a fluffy white solid (443 mg, 85%). MW 656.56 ($C_{20}H_{33}O_{17}N_4SNa$).

Example 21 -- Preparation of 2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl amine

Procedure A.

2-Acetamido-2-deoxy-3-O-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-fucopyransoyl)-4-*O*-[4,6-*O*-*p*-methoxybenzylidene-3-*O*-sulfo-β-D-galactopyranosyl]-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide (compound **110** – 500 mg, 0.398 mmol) was weighed into a reaction flask. The vessel was then flushed with nitrogen and 500 mg of 5% palladium on charcoal was added to the flask. Dry methanol (6.0 mL) was syringed into the flask followed by 4.0 mL of a 0.37% methanolic hydrochloric acid solution. The mixture was then purged with hydrogen gas and remained under hydrogen atmosphere for four hours at room temperature. At this time, another 1.0 mL of acidic solution was added to the reaction (total mmols of HCl = 0.51, 1.28 eq.). The reaction was stirred an additional four hours and quenched by the addition of pyridine until the pH of the solution was basic. The reaction solution was then filtered and the catalyst washed well with water and methanol. The solution was concentrated on a rotary evaporator (water bath < 25°C) and purified by column chromatography (Iatrobeads 85:10:5 isopropanol:water:ammonia was the eluant). The total time the product was in contact with silica gel was three hours. The product was collected, pyridine added to the collection flask and concentrated as noted above. The product was then loaded onto 8 g of Na^+ analytical grade ion exchange resin and lyophilized to yield 191 mg (75%) of a fluffy white powder. MW of sodium salt 630.55 ($C_{20}H_{35}O_{17}N_2SNa$). NMR revealed the product was 20-30% decomposed.

It was found that the lifetime of the product could be enhanced by using the reaction mixture immediately after hydrogenation. MW (pyridinium salt) 687.68 ($C_{25}H_{41}O_{17}N_3S^-$).

Procedure B.

2-Acetamido-2-deoxy-(3-*O*-α-L-fucopyranosyl]-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranosyl azide (compound **111** -- 150 mg, 0.22 mmol) and 5% palladium on charcoal (200 mg) were added to a reaction vessel and purged with nitrogen. Dry methanol (10 mL) was syringed into the flask and the reaction contents were cooled to 0°C. The reaction vessel was then purged with hydrogen gas and stirred at 0°C for two hours. The reaction was quenched by the addition of pyridine, filtered and concentrated. Caution was used to maintain all procedures at or below room temperature to reduce risk of product decomposition. The crude product was used directly for further chemical manipulations without any characterization. Tlc of the reaction mixture when complete indicates a major product spot at Rf = 0.30 (75:20:5 isopropanol:water:ammonia) with a minor spot at Rf = 0.20 (approximately 20% as intense as the product spot by sulfuric acid charring).

Example 22 -- Preparation of 2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl benzamide sodium salt

The crude glycosyl amine (190 mg, 0.28 mmol - pyridinium salt from Example 21 -- Procedure A) was dissolved in 8.5 mL of methanol and 3.0 mL of a saturated solution of sodium bicarbonate. The reaction mixture was cooled to 0°C and benzoyl chloride (0.175 mL, 1.5 mmol, 5.4 eq.) was syringed into the mixture all at once. The reaction was complete within 5 minutes, as indicated by an increase in Rf on tlc (Rf starting material = 0.31, product = 0.53 in 75:20:5 isopropanol:water:ammonia). A few drops of pyridine were added to the reaction mixture and it was concentrated under reduced pressure. The product was isolated by

Iatrobead column chromatography using 70:30:3:1 dichloromethane: methanol:water:pyridine as eluent. Concentration of the product was followed by ion exchange chromatography using 10 g of analytical grade Na^+ resin. Lyophilization yielded 122 mg of product (59%) as a fluffy white powder. MW 734.66 ($C_{27}H_{39}O_{18}N_2SNa$).

Example 23 -- Preparation of 2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl *p*-nitrobenzamide sodium salt

The crude glycosyl amine (335 mg, 0.49 mmol - pyridinium salt from Example 21 -- Procedure A) was dissolved in 15 mL of methanol and 5 mL of a saturated solution of sodium bicarbonate. The reaction mixture was cooled to 0°C and *p*-nitrobenzoyl chloride (698 mg, 3.8 mmol, 7.7 eq.) was added to the reaction mixture. Acetone (1 mL) was syringed into the mixture to enhance the solubility of the acid chloride. The reaction was stirred overnight at room temperature. A few drops of pyridine was added to the reaction and the mixture was concentrated. The product was purified as described for the preparation of the benzamido product (Example 22). The chromatography eluent was 70:30:3:1 dichloromethane:methanol:water:pyridine. The product, 88 mg (23%), was obtained as a fluffy white solid. MW 779.66 ($C_{27}H_{38}O_{20}N_3SNa$).

Example 24 -- Preparation of 2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl butyramide sodium salt

The crude glycosyl amine (292 mg, 0.42 mmol - pyridinium salt from Example 21 -- Procedure A) was dissolved in 12 mL of methanol and 4 mL of a saturated solution of sodium bicarbonate. The reaction mixture was cooled to 0°C and butyric anhydride (0.39 mL, 2.4 mmol, 5.7 eq.) was added to the reaction mixture. After 5 minutes, a few drops of pyridine was added to the reaction and the mixture was concentrated. The product was purified as described for the preparation of the benzamido

product (Example 22). The chromatography eluent was 70:30:2:1 dichloromethane:methanol:water:pyridine. A second column using 85:10:5 isopropanol:water:ammonia was used to repurify the product. The product, 90 mg (30%), was obtained as a fluffy white solid. MW 700.65 ($C_{24}H_{41}O_{18}N_2SNa$).

Example 25 -- Preparation of 2-Acetamido-2-deoxy-3-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl acetamide sodium salt

The crude glycosyl amine (135 mg, 0.20 mmol - pyridinium salt from Example 21 -- Procedure A) was dissolved in 4.5 mL of methanol and 1.5 mL of a saturated solution of sodium bicarbonate. The reaction mixture was cooled to 0°C and acetic anhydride (0.095 mL, 1.0 mmol, 5 eq.) was added to the reaction mixture. After several hours, a few drops of pyridine were added to the reaction and the mixture was concentrated. The product was purified as described for the preparation of the benzamido product (Example 22). The chromatography eluent was 85:11:4 isopropanol:water:ammonia. A second column was used to repurify the product (eluent was 70:30:2.5:1 dichloromethane:methanol:water:pyridine). The product, 118 mg (88%), was obtained as a fluffy white solid. MW 672.59 ($C_{22}H_{37}O_{18}N_2SNa$).

Example 26 -- Preparation of 2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl stearamide sodium salt

Stearic acid (564 mg, 2.0 mmol, 9 eq.) and dicyclohexylcarbodiimide (590 mg, 2.9 mmol, 13 eq.) were weighed into a reaction flask. Methanol (70 mL) was added to the flask and the mixture was stirred at 0°C for 30 minutes. The crude glycosyl amine (158 mg, 0.23 mmol, 1 eq. -- pyridinium salt from Example 21 -- procedure B) was dissolved in 2 x 5 mL of methanol and added to the reaction flask. The reaction was stirred overnight at room temperature and then a few drops of pyridine were added

to the reaction flask. The mixture was then concentrated and purified as described for the preparation of the benzamido product (Example 22). The chromatography eluent was 80:20:2 dichloromethane:methanol:pyridine. The product, 62 mg (30%), was obtained as a fluffy white solid. MW 897.02 ($C_{38}H_{69}O_{18}N_2SNa$).

Example 27 -- Preparation of 2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-[3-*O*-sulfo-β-D-galactopyranosyl]-β-D-glucopyranosyl L-serine sodium salt

N-Benzyloxycarbonyl-L-serine (450 mg, 1.9 mmol, 9 eq.) and EDC (560 mg, 2.9 mmol, 14 eq.) were weighed into a reaction flask. Methanol (65 mL) was added to the flask and the mixture was stirred at 0°C for 30 minutes. The crude glycosyl amine (142 mg, 0.21 mmol, 1eq. -- pyridinium salt from Example 21 procedure b) was dissolved in 2 x 5 mL of methanol and added to the reaction flask. The reaction was stirred overnight at room temperature and then a few drops of pyridine were added to the reaction flask. The mixture was then concentrated and purified as described for the preparation of the benzamido product (Example 22). The chromatography eluent was 70:30:2:0.1 dichloromethane:methanol:water: pyridine. The benzyloxycarbonyl protected product, 115 mg (64%), was obtained as a fluffy white solid. MW 851.77 ($C_{31}H_{46}O_{21}N_3SNa$).

The benzyloxycarbonyl protected product (115 mg, 0.13 mmol) was added to a reaction flask followed by 200 mg of 5% palladium on charcoal. The flask was argon purged and then methanol (10 mL) was added. The reaction flask was then purged with hydrogen for one hour at room temperature. Tlc of the reaction after one hour indicated the reaction was complete. Several drops of pyridine were added to the reaction and the catalyst was removed by filtration. The filtrate was concentrated and purified as described for the preparation of the benzamido product (Example 22). The chromatography eluent was 65:35:4:0.1 dichloromethane:methanol:water:pyridine. The glucopyranosyl serine

product was obtained after lyophilization as a fluffy white solid (60 mg, 62%). MW 717.64 ($C_{23}H_{40}O_{19}N_3SNa$).

Example 28 -- Preparation of 8-methoxycarbonyloctyl-2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-fucopyranosyl)-4-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside sodium salt (compound 212)

The synthesis of this compound is illustrated in Figure 12.

Step A -- Preparation of Methyl 2-O-benzyl-L-fucopyranoside, compound 201

The title compound, compound **201**, was prepared according to Deter-Jusynski, et al.[36]

Step B -- Preparation of Methyl 2,3-di-O-benzyl-L-fucopyranoside (compound 202)

A mixture of compound **201** (7 g, 26.089 mmol), di-*n*-butyltin oxide (7 g, 28.12 mmol) in anhydrous benzene (300 mL) was boiled under reflux with azetropic removal of water for 6 hours and then concentrated to 50 mL. Tetrabutylammonium fluoride monohydrate (9.8 g, 37.48 mmol), 4A molecular sieves (23 g) and benzyl bromide (9.25 mL, 77.77 mmol) were then added and the mixture was kept at room temperature overnight. Column chromatography on silica gel (hexane-ethyl acetate, 2:1) gave compound **202** (9.1 g, 97%).

Step C -- Preparation of Methyl 2,3-di-O-benzyl-4-O-(methylthio)thiocarbonyl-L-fucopyranoside (compound 203)

Sodium hydride (428 mg, 60% in mineral oil, 10.7 mmol) and imidazole (10 mg) were added to compound **202** (750 mg, 2.092 mmol) in THF (16 mL). The reaction mixture was heated at 50°C for 0.5 hours and

carbon disulfide (16 mL) was added. In another 1 hour, methyl iodide (16 mL) was added. Heating was continued for 1 more hour and the mixture was cooled to room temperature and methanol was added. Concentration and then column chromatography (hexane-ethyl acetate, 3:1) gave compound **203** (850 mg, 90%).

Step D -- Preparation of Methyl 2,3-di-O-benzyl-4-O-(imidazole)thiocarbonyl-L-fucopyranoside (compound 204)

A mixture of compound **202** (8 g, 22.32 mmol) and 1,1'-thiocarbonyldiimidazole (9 g, 50.50 mmol) in 1,2-dichloroethane (100 mL) was refluxed for 4 hours and then concentrated. Column chromatography of the residue (hexane-ethyl acetate, 2:1, 1:1) yielded compound **204** (10.1 g, 96%).

Step E -- Preparation of Methyl 2,3-di-O-benzyl-4-deoxy-L-fucopyranoside (compound 205)

Preparation A (from compound **203**)

Compound **203** (850 mg, 1.895 mmol) in anhydrous toluene (20 mL) was heated at 80°C. AIBN (350 mg, 2.142 mmol) was added followed by tri-butyltin hydride (8 mL, 29.74 mmol). Heating was continued for 2 hours and the mixture was concentrated and chromatographed (hexane-ethyl acetate, 3:1) to give compound **205** (620 mg, 95%).

Preparation B (from compound **204**)

Compound **204** (10 g, 21.3 mmol) in anhydrous toluene (160 mL) was heated at 100°C. AIBN (2.6 g, 15.912 mmol) and tributyltin hydride (50 g, 171.8 mmol) were then added. Heating was continued for 2 hours and the mixture was concentrated and chromatographed (hexane-ethyl acetate, 6:1) to give compound **205** (5.4 g, 74%).

Step F -- Preparation of 2,3-Di-O-benzyl-4-deoxy-L-fucopyranose (compound 206)

Compound **205** (10 g, 29.2 mmol) in glacial acetic acid (180 mL) and 6 N HCl (36 mL) was heated at 65°C for 3 hours and evaporated. The crude product, compound **206**, was used in the next reaction without purification.

Step G -- Preparation of *p*-Chlorophenyl 2,3-di-O-benzyl-4-deoxy-β-L-thiofucopyranoside (compound 207)

A mixture of compound **206** (29.2 mmol), anhydrous NaOAc (2.5 g, 30.48 mmol) and acetic anhydride (53 mL) in 1,2-dichloroethane (90 mL) was heated at 60°C for 3 hours and cooled to room temperature. Water was added and the organic layer was separated and washed with water and aqueous sodium bicarbonate. After concentration, the dried residue was dissolved in dichloromethane (100 mL) and the mixture was cooled at 0°C. *p*-Chlorothiophenol (4.5 g, 31.116 mmol) was added followed by BF_3-ether (7.3 mL). The reaction mixture was allowed to reached room temperature and kept there for 1.5 hours. It was then poured into ice-$NaHCO_3$ and extracted with dichloromethane. Column chromatography (hexane-ethyl acetate, 6:1) of the residue on evaporation gave compound **207** (10.5 g, 79% 3-steps).

Step H -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-4-O-(2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (compound 208)

Compound **208** was prepared according to the procedures provided in Internatinal Patent Application Serial No. PCT/US93/04909 which is incorporated herein by reference in its entirety.

Step I -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(2,3-di-O-benzyl-4-deoxy-α-L-fucopyranosyl)-4-O-(2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (compound 209)

DMF (3 mL) and tetraethylammonium bromide (768 mg, 3.654 mmol) were added to a suspension of copper bromide (4.17 g, 18.669 mmol) and 4A molecular sieves (7.2 g) in dichloromethane (6 mL). After stirring at room temperature for 0.5 hours, a solution of compound **208** (3.6 g, 3.83 mmol) in dichloromethane (7 mL) was added followed by compound **207** (4 g, 8.79 mmol) in dichloromethane (6 mL). The mixture was then kept in the dark for 16 hours and pyridine (1 mL) was added. The resulting solution was filtered through Celite, concentrated and column chromatographed (hexane-acetone, 5:2) to give compound **209** (3.5 g, 73%).

Step J -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,3-di-O-benzyl-4-deoxy-α-L-fucopyranosyl)-4-O-(4,6-O-benzylidene-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (compound 210)

Compound **209** (3.3 g, 2.64 mmol) was treated with 0.06 N methanolic sodium methoxide (90 mL) for 16 hours. It was neutralized by Amberlite IR 120 (H^+), filtered and evaporated. Column chromatography of the residue (hexane-acetone, 2:1) provided for compound **210** (2.5 g, 91%).

Step K -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,3-di-O-benzyl-4-deoxy-α-L-fucopyranosyl)-4-O-(4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside sodium salt (compound 211)

Sulfur trioxide-pyridine complex (687 mg, 4.32 mmol) was added to compound **210** (2.5 g, 2.4 mmol) in pyridine (16 mL) at 0°C. The reaction mixture was allowed to reach room temperature and more sulfur

tioxide-pyridine complex (300 mg, 1.885 mmol) was added. Stirring was continued for 1 more hour and methanol was added. After evaporation of the solvent, the residue was applied to a column of silica gel (methanol-dichloromethane, 1:9, 0.1% pyridine). The product obtained was then passed through a column of Amberlite IR-120 Na^+ (MeOH) to give compound 211 (2.2 g, 80%).

Step L -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-fucopyranosyl)-4-O-(3-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside sodium salt (compound 212)

Compound 211 (1.5 g, 1.311 mmol) and palladium on carbon (5%, 2.2 g) in MeOH (80 mL) were hydrogenated for 3 hours and pyridine was added. After filtration of the catalyst and evaporation of the solvent, the crude product was purified by chromatography on silica gel (dichloromethane-methanol-water, 80:20:2, 0.2% pyridine) and then a column of Bio-Rex® 70 (Na^+ form) to provide for compound 212 (750 mg, 73%).

Example 29 -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-3-O-(4-O-sulfo-α-L-fucopyranosyl)-4-O-(3-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside sodium salt (compound 224)

The synthesis of this compound is illustrated in Figure 13.

Step A -- Preparation of *p*-Chlorophenyl 3,4-O-benzylidene-β-L-thiofucopyranoside (compound 214)

A mixture of compound 213 (1.8 g, 6.19 mmol), p-toluenesulfonic acid (20 mg) and α,α-dimethoxytoluene (1.6 mL, 10.66 mmol) in acetonitrile (10 mL) was kept at room temperature overnight and concentrated to 6 mL. It was then heated at 55°C for 2 hours and

neutralized by triethylamine and evaporated to give crude compound **214** which was used in the next reaction.

Step B -- Preparation of *p*-Chlorophenyl 2-O-benzyl-3,4-O-benzylidene-β-L-thiofucopyranoside (compound 215)

Sodium hydride (383 mg, 60% in mineral oil, 9.575 mmol) was added in small portions to compound **214** (3.1 mmol) in DMF (4 mL) which was cooled in dry ice. After 0.5 hours, benzyl bromide (1.2 mL) was added and the reaction mixture was allowed to reach room temperature and kept there for 2 hours. Methanol was added to destroy the remaining NaH and the solvent were then removed by evaporation. The residue was taken up in dichloromethane, filtered through ceilite and evaporated to leave crude compound **215** as a syrup.

Step C -- Preparation of *p*-Chlorophenyl 2-O-benzyl-β-L-thiofucopyranoside (compound 216)

Compound **215** (3.0 mmol) in 80% acetic acid (20 mL) was heated at 50°C for 2 hours. It was evaporated and coevaporated with toluene. Chromatography of the residue (hexane-ethyl acetate, 1:1) provided compound **216** (720 mg, 63%, 3 steps).

Step D -- Preparation of *p*-Chlorophenyl 2,3-di-O-benzyl-β-L-thiofucopyranoside (compound 217)

A mixture of compound **216** (700 mg, 1.838 mmol) and di-*n*-butyltin oxide (495 mg, 1.988 mmol) in benzene (40 mL) was boiled under reflux with azetropic removal of water for 6 hours and then concentrated to 5 mL. Tetrabutylammonium fluoride monohydrate (700 mg, 2.67 mmol), 4A molecular sieves (1.7 g) and benzyl bromide (0.7 mL, 5.89 mmol) were then added and the mixture was kept at room temperature overnight. Column chromatography on silica gel (hexane-ethyl acetate, 3:1) gave compound **217** (810 mg, 93%).

Step E -- Preparation of *p*-Chlorophenyl 2,3-di-O-benzyl-4-O-(4-methoxybenzyl)-β-L-thiofucopyranoside (compound 218)

A solution of compound **217** (660 mg, 1.401 mmol) in DMF (6 mL) was cooled in dry ice and sodium hydride (584 mg, 60% in mineral oil, 14.6 mmol) was added in small portions. After 0.5 hours, *p*-methoxybenzyl chloride (1.83 mL, 13.50 mmol) was added and the reaction mixture was then kept at room temperature for 3 hours. Methanol was then added. After evaporation, the residue was applied to a column of silica gel (hexane-ethyl acetate, 10:1) and the appropriate fractions collected to provide for compound **218** (605 mg, 73%).

Step F -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(2,3-di-O-benzyl-4-O-(4-methoxybenzyl)-α-L-fucopyranosyl)-4-O-(2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (compound 219)

DMF (0.39 mL) and tetraethylammonium bromide (106 mg, 0.505 mmol) were added to a suspension of copper bromide (552 mg, 2.473 mmol) and 4A molecular sieves (880 mg) in dichloromethane (1 mL). After stirring at room temperature for 0.5 hours, a solution of compound **208** (490 mg, 0.521 mmol) in dichloromethane (1 mL) was added followed by compound **218** (440 mg, 0.745 mmol) in dichloromethane (1 mL). The mixture was then kept in the dark for 24 hours and pyridine (0.3 mL) was added. It was filtered through celite, concentrated and column chromatographed (hexane-acetone, 5:2) to give compound **219** (680 mg, 94%).

Step G -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(2,3-di-O-benzyl-4-O-(4-methoxybenzyl)-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-β-D-glucopyranoside (compound 220)

Compound **219** (670 mg, 0.483 mmol) was treated with 0.06 N methanolic sodium methoxide (15 mL) for 16 hours. It was neutralized by Amberlite IR 120 (H^+), filtered and evaporated. Column chromatography of the residue (hexane-acetone, 1:1) provided compound **220** (550 mg, 97%).

Step H -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,3-di-O-benzyl-4-O-(4-methoxybenzyl)-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (compound 221)

Sulfur trioxide pyridine complex (119 mg, 0.747 mmol) was added to compound **220** (550 mg, 0.467 mmol) in pyridine (2 mL) at 0°C. The reaction mixture was allowed to reach room temperature. Stirring was continued for 1 more hour and methanol was added. After evaporation of the solvent, the crude product, compound **221**, was used in the next reaction.

Step I -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,3-di-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (compound 222)

A mixture of compound **221** (0.336 mmol) and CAN (460 mg, 0.839 mmol) in acetonitrile-water (9:1, 10 mL) was kept at room temperature for 1 hour and pyridine (0.6 mL) was added followed by dichloromethane. The organic layer was separated and washed with water. After evaporation of the solvent, the residue was applied to a column of silica gel (dichloromethane-methanol, 85:15, 0.2% pyridine) to provide for compound **222** (350 mg, 87%, 2 steps).

Step J -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,3-di-O-benzyl-4-O-sulfo-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D- galactopyranosyl]-2-deoxy-β-D-glucopyranoside disodium salt (compound 223)

Sulfur trioxide-pyridine complex (80 mg, 0.502 mmol) was added to compound 222 (350 mg, 0.291 mmol) in pyridine (2.4 mL) at 0°C. The reaction mixture was allowed to reach room temperature and more sulfur trioxide-pyridine complex (120 mg, 0.753 mmol) was added in small portions. Stirring was continued for 3 hours and methanol was added. After evaporation of the solvent, the residue was applied to a column of silica gel (water-methanol-dichloromethane, 2:20:80, 0.2% pyridine used as eluent). The product obtained was then passed through a column of Amberlite R-120 Na^+ (MeOH) to give compound 223 (180 mg, 49%).

Step K -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-3-O-(4-O-sulfo-α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-β-D-glucopyranoside disodium salt (compound 224)

Compound 223 (150 mg, 0.119 mmol) and palladium on carbon (5%, 280 mg) in MeOH (8 mL) were hydrogenated for 2 hours and pyridine (0.8 mL) was added. After filtration of the catalyst and evaporation of the solvent, the crude product was purified by chromatography on silica gel (dichloromethane-methanol-water, 70:30:3, 0.2% pyridine) and then a column of Bio-Rex® 70 (Na^+ form) to provide for compound 224 (81 mg, 76%).

Example 30 -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-3-O-(3-O-sulfo-α-L-fucopyranosyl)-4-O-(3-O-sulfo-β-D-galactopyranosyl)-β-D-glucopyranoside disodium salt (compound 232)

The synthesis of compound 232 is illustrated in Figure 14.

Step A -- Preparation of *p*-Chlorophenyl 2-O-benzyl-3-O-(4-methoxybenzyl)-β-L-thiofucopyranoside (compound 225)

A mixture of compound **216** (660 mg, 1.732 mmol) and di-*n*-butyltin oxide (495 mg, 1.988 mmol) in benzene (60 mL) was boiled under reflux with azetropic removal of water for 6 hours and then concentrated to 10 mL. Tetrabutylammonium fluoride monohydrate (660 mg, 2.524 mmol), 4A molecular sieves (1.7 g) and 4-methoxybenzyl chloride (0.77 mL, 5.66 mmol) were then added and the mixture was kept at room temperature overnight. Column chromatography on silica gel (hexane-ethyl acetate, 3:1) gave compound **225** (770 mg, 89%).

Step B -- Preparation of *p*-Chlorophenyl 2,4-di-O-benzyl-3-O-(4-methoxybenzyl)-β-L-thiofucopyranoside (compound 226)

A solution of compound **225** (750 mg, 1.497 mmol) in DMF (5 mL) was cooled in dry ice and sodium hydride (600 mg, 60% in mineral oil, 15 mmol) was added in small portions. After 0.5 hours, benzyl bromide (1.7 mL, 14 mmol) was added and the reaction mixture was then kept at room temperature for 2 hours. Methanol was added. After evaporation, the residue was applied to a column of silica gel (hexane-ethyl acetate, 6:1) to provide for compound **226** (705 mg, 80%).

Step C -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(2,4-di-O-benzyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl)-4-O-[2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosyl]-β-D-glucopyranoside (compound 227)

DMF (0.34 mL) and tetraethylammonium bromide (106 mg, 0.504 mmol) were added to a suspension of copper bromide (477 mg, 2.137 mmol) and 4A molecular sieves (760 mg) in dichloromethane (1 mL). After stirring at room temperature for 0.5 hours, a solution of compound **208** (300 mg, 0.319 mmol) in dichloromethane (1.5 mL) was added

followed by compound **226** (380 mg, 0.642 mmol) in dichloromethane (1.5 mL). The mixture was then kept in the dark for 5 hours and pyridine (0.5 mL) was added. It was filtered through Celite, concentrated and column chromatographed (hexane-acetone, 5:2) to give compound **227** (360 mg, 81%).

Step D -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,4-di-O-benzyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (compound 228)

Compound **227** (330 mg, 0.237 mmol) was treated with 0.06 N methanolic sodium methoxide (8 mL) for 16 hours. It was neutralized by Amberlite IR 120 (H^+), filtered and evaporated. Column chromatography of the residue (hexane-acetone, 1:1) provided for compound **228** (255 mg, 91%).

Step E -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,4-di-O-benzyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (compound 229)

Sulfur trioxide-pyridine complex (50 mg, 0.314 mmol) was added to compound **228** (245 mg, 0.207 mmol) in pyridine (1.6 mL) at 0°C. The reaction mixture was allowed to reach room temperature and more sulfur trioxide-pyridine complex (20 mg) was added. Stirring was continued for 0.5 more hours and methanol was added. After evaporation of the solvent, the crude product, compound **229**, was used in the next reaction.

Step F -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,4-di-O-benzyl-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (compound 230)

A mixture of compound **229** (0.207 mmol) and CAN (400 mg, 0.730 mmol) in acetonitrile-water (9:1, 10 mL) was kept at room temperature for 3 hours and pyridine (0.6 mL) was added followed by dichloromethane. The organic layer was separated and washed with water. After evaporation of the solvent, the residue was applied to a column of silica gel (dichloromethane-methanol, 85:15, 0.2% pyridine) to yield compound **230** (190 mg, 76%, 2 steps).

Step G -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyl-3-O-(2,4-di-O-benzyl-3-O-sulfo-α-L-fucopyranosyl)-4-O-[4,6-O-benzylidene-3-O-sulfo-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside disodium salt (compound 231)

Sulfur trioxide-pyridine complex (80 mg, 0.502 mmol) was added to compound **230** (140 mg, 0.116 mmol) in pyridine (1 mL) at 0°C. The reaction mixture was allowed to reach room temperature and more sulfur trioxide- pyridine complex (70 mg) was added in small portions. Stirring was continued for 1.5 hours and methanol was added. After evaporation of the solvent, the residue was applied to a column of silica gel (methanol-dichloromethane, 15:85, 0.2% pyridine). The product obtained was then passed through a column of Amberlite IR-120 Na^+ (MeOH) to give compound **231** (110 mg, 75%).

Step H -- Preparation of 8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-3-O-(3-O-sulfo-α-L-fucopyranosyl)-4-O-[3-O-sulfo-β-D-galactopyranosyl]-β-D-glucopyranoside disodium salt (compound 232)

Compound **231** (110 mg, 0.087 mmol) and palladium on carbon (5%, 150 mg) in MeOH (4 mL) were hydrogenated for 2 hours and pyridine (2 mL) was added. After filtration of the catalyst and evaporation of the solvent, the crude product was purified by chromatography on silica gel (dichloromethane-methanol-water, 70:30:3, 0.2% pyridine) and then a